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Original article

Upper respiratory tract SARS-CoV-2 RNA loads in symptomatic and asymptomatic children and adults

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ABSTRACT

Objectives: Studies comparing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA load in the upper respiratory tract (URT) between children and adults—who either presented with coronavirus disease 2019 (COVID-19) or were asymptomatic—have yielded inconsistent results. Here, we conducted a retrospective, single-centre study to address this issue.

Patients and methods: Included were 1184 consecutive subjects (256 children and 928 adults) testing positive for SARS-CoV-2 RNA in nasopharyngeal exudates (NPs); of these, 424 (121 children and 303 adults) had COVID-19 and 760 (135 children and 625 adults) were asymptomatic close contacts of COVID-19 patients. SARS-CoV-2 RNA testing was carried out using the TaqPath COVID-19 Combo Kit (Thermo Fisher Scientific, MS, USA). The AMPLIRUN® TOTAL SARS-CoV-2 RNA Control (Viracell SA, Granada, Spain) was used for estimating SARS-CoV-2 RNA loads (in copies/mL). SARS-CoV-2 RNA loads at the time of laboratory diagnosis (single specimen/patient) were used for comparison purposes.

Results: Median initial SARS-CoV-2 RNA load was lower (p 0.094) in children (6.98 log₁₀ copies/mL, range 3.0–11.7) than in adults (7.14 log₁₀ copies/mL, range 2.2–13.4) with COVID-19. As for asymptomatic individuals, median SARS-CoV-2 RNA load was comparable (p 0.97) in children (6.20 log₁₀ copies/mL, range 1.8–11.6) and adults (6.48 log₁₀ copies/mL, range 1.9–11.8). Children with COVID-19 symptoms displayed SARS-CoV-2 RNA loads (6.98 log₁₀ copies/mL, range 3.0–11.7) comparable to those of their asymptomatic counterparts (6.20 log₁₀ copies/mL, range 1.8–11.6) (p 0.61). Meanwhile in adults, median SARS-CoV-2 RNA load was significantly higher in symptomatic (7.14 log₁₀ copies/mL, range 2.2–13.4) than in asymptomatic subjects (6.48 log₁₀ copies/mL, range 1.9–11.8) (p < 0.001). Overall, the observed URT SARS-CoV-2 RNA clearance rate was faster in children than in adults.

Conclusions: Based on viral load data at the time of diagnosis, our results suggest that SARS-CoV-2-infected children, with or without COVID-19, may display NP viral loads of comparable magnitude to those found in their adult counterparts. However, children may have shorter viral shedding than adults.

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Introduction

An increasing body of evidence suggests that children are less susceptible to infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and tend to develop milder forms of coronavirus disease 2019 (COVID-19) than adults [1]. Nevertheless,

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whether children—either symptomatic or asymptomatic—play a major role in community transmission of SARS-CoV-2 compared to adults remains unclear [1]. There is a consistent direct correlation between the magnitude of SARS-CoV-2 RNA load in the upper respiratory tract (URT) (probability of recovering live virus in cell culture) and contagiousness in both adults and children [2–5]. Supporting this assumption, transmission risk was recently shown to be strongly associated with initial SARS-CoV-2 RNA levels of index cases [6]. There is contradictory information on how SARS-CoV-2 RNA load in the URT compares between children and adults [7–11], whether the viral load in paediatric subjects differs across ages [9,10], and whether dissimilarities in the dynamics of SARS-CoV-2 shedding in the URT exist between symptomatic and asymptomatic children [12–14]. Elucidation of these questions is important in trying to establish the relative transmission risk between adults and children, although it should be kept in mind that, in addition to URT viral load, other factors—such as the ability to release aerosols, the nature of symptoms and social mixing patterns—may modulate contagiousness. Here, to gain further insight into these issues, we conducted a retrospective, single-centre study involving a substantial cohort of SARS-CoV-2-infected children and adults who were either asymptomatic or symptomatic, non-hospitalized cases.

Methods

Patients and specimens

A convenient sample was used consisting of 1184 consecutive subjects (256 children and 928 adults, 520 males and 664 females) testing positive for SARS-CoV-2 RNA in nasopharyngeal exudates (NPs) between June 2020 and January 2021. The subjects either presented with symptoms compatible with COVID-19, including one or more of the following: fever, dry cough, rhinorrhoea, dyspnoea, myalgia, fatigue, anosmia, ageusia, odynophagia, diarrhoea, conjunctivitis, and cephalgia (none requiring hospitalization) or they were asymptomatic close contacts of COVID-19 patients, as previously defined [15], at the time of RT-PCR testing. A total of 967 participants (154 children and 813 adults) were sampled at primary health centres belonging to the Health Department Clínico-Malvarrosa, Valencia (Spain), while 217 (102 children and 115 adults) were sampled at the Emergency Department of Hospital Clínico Universitario of Valencia. The current study was approved by the Research Ethics Committee of Hospital Clínico Universitario INCLIVA (March 2020). The requirement to obtain informed consent was waived by the Ethics Committee.

SARS-CoV-2 RNA testing

NPs were collected by trained nurses at sampling sites and were placed in 3 mL of universal transport medium (Becton Dickinson, Sparks, MD, USA). RT-PCRs were carried out at the Microbiology Service of Hospital Clínico Universitario within 24 h of specimen collection. The TaqPath COVID-19 Combo Kit (Thermo Fisher Scientific, MS, USA), which targets SARS-CoV-2 ORF1ab, N and S genes, was used following RNA extraction carried out using the Applied Biosystems™ MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kits coupled with Thermo Scientific™ KingFisher Flex automated instrument. The AMPLIRUN® TOTAL SARS-CoV-2 RNA Control (Viracell SA, Granada, Spain) was used as the reference material for estimating SARS-CoV-2 RNA load (in copies/mL, taking RT-PCR CTs for the N gene) [16,17].

RT-PCR β -glucuronidase RNA testing

We amplified the β -glucuronidase (GUSB) housekeeping gene to assess specimen cellularity in selected specimens, following a previously published protocol [18]. In brief, RNA was extracted from the NP using the DSP virus Pathogen Minikit on the QiaSymphony Robot instruments (Qiagen, Valencia, CA), reverse-transcribed to complementary DNA, and subsequently amplified by using the HEQC one-step kit (Seqplexing, Valencia, Spain) in the LightCycler 480 Real-Time PCR System Version II (Roche Diagnostics, Pleasanton).

Statistical methods

Differences between medians across groups were compared in a pairwise fashion using the non-parametric Mann–Whitney U-test, given that SARS-CoV-2 RNA loads were non-normally distributed. Spearman's rank test was used to test the association between age and SARS-CoV-2 RNA load. Two-sided exact p-values were reported; $p < 0.05$ was considered statistically significant. The analyses were performed using SPSS version 20.0 (SPSS, Chicago, IL, USA).

Results

Study groups

Participants were paediatric individuals (≤ 18 years, $n = 256$, 21.6%) aged a median of 12 years (range 0–18 years), or adults (> 18 years, $n = 928$, 78.3%) aged a median of 37 years (range 19–93 years). A total of 424 subjects (121 children, 303 adults) presented with symptoms compatible with COVID-19. Specimen collection was carried out at a median of 2 days (range 0–10 days) and a median of 3 days (range 0–10 days) after the onset of symptoms in children and adults, respectively. A total of 760 participants (135 children and 625 adults) were close contacts of COVID-19 patients and asymptomatic at the time of testing positive by RT-PCR for the first time. No information was available on our database regarding whether these latter individuals were household or non-household contacts or whether or not they developed symptoms later on. NP specimen collection in contacts was prescribed at the discretion of either the physician in charge of the index case or local health authorities, and was performed at a median of 7 days (range 1–10 days) after diagnosis of the presumed index case, in both children and adults. Initial SARS-CoV-2 RNA loads were used throughout the current study for comparison purposes.

SARS-CoV-2 RNA load in paediatric and adult COVID-19 patients

We first compared initial SARS-CoV-2 RNA load in NP from symptomatic paediatric and adult patients. The data are shown in Fig. 1A. The range of SARS-CoV-2 RNA loads appeared comparable between children and adults. Median viral RNA load in children (6.98 \log_{10} copies/mL) was lower than that in adults (7.14 \log_{10} copies/mL), although the difference did not reach statistical significance ($p = 0.094$).

We next compared the initial SARS-CoV-2 RNA load in children and adults by time of NP sampling after symptom onset. SARS-CoV-2 RNA load peaks soon after or at the time of symptom onset, or less commonly at day 3–5 of illness (see Cevik et al. for a systematic review and meta-analysis [19]). We arbitrarily split each patient group into two subgroups (within 2 days/ ≥ 3 days after symptom onset). SARS-CoV-2 RNA load was significantly higher in NP specimens collected within 2 days after onset of symptoms than in those obtained later on, in both children (median 7.46 \log_{10} copies/mL versus 5.17 \log_{10} copies/mL, $p < 0.001$) and adults (7.81 \log_{10}

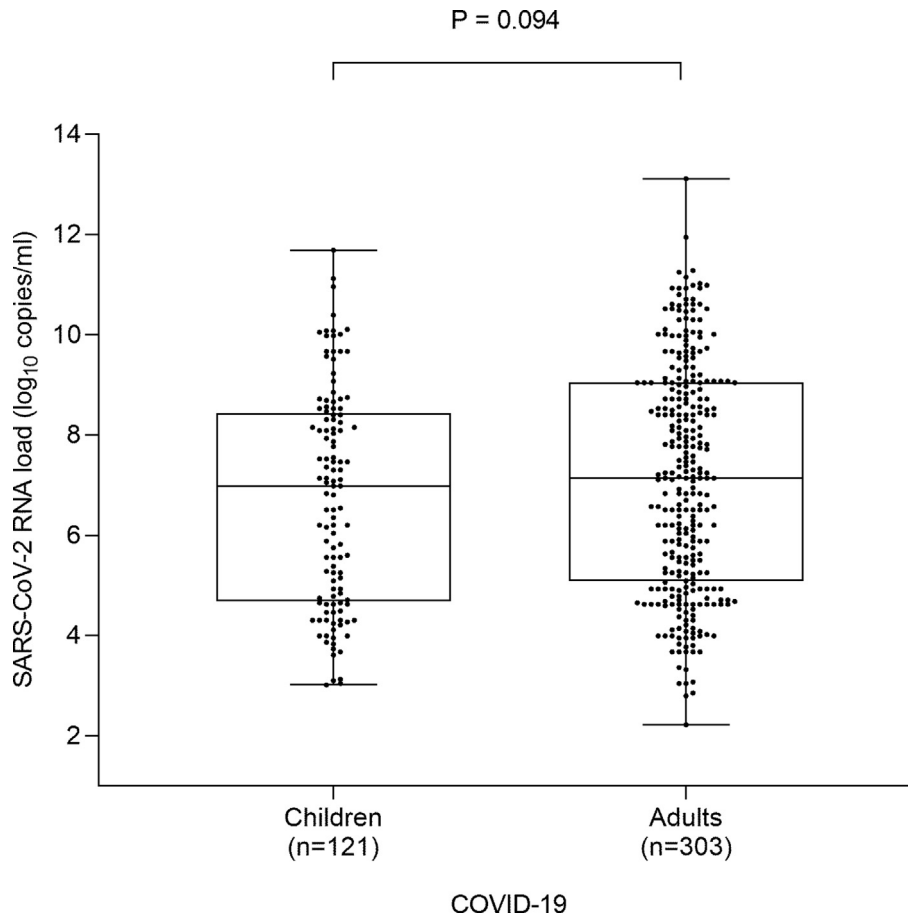


Fig. 1. Overall initial severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA loads in nasopharyngeal specimens from children and adults with coronavirus disease 2019 (COVID-19). Medians are indicated by midlines; the top and bottom edges of boxes represent the interquartile range (IQR). Whiskers indicate the upper and lower values. The number of patients in each group as well as p values for comparisons between groups (median SARS-CoV-2 RNA levels) are shown.

copies/mL versus 6.45 log₁₀ copies/mL, p 0.002) (Fig. 2). Interestingly, SARS-CoV-2 RNA load measured within 2 days after symptom onset was comparable (p 0.26) between children and adults, whereas those determined at later times (≥ 3 days) were significantly lower in children (p 0.002).

Finally, as shown in Fig. 3A and B, no correlation was found between initial SARS-CoV-2 RNA loads and patient age, for either children (ρ 0.008, p 0.93) or adults (ρ 0.005, p 0.92). Further results across age groups are shown in the [Supplementary Material](#).

SARS-CoV-2 RNA load in asymptomatic children and adults

A wide range of SARS-CoV-2 RNA loads were detected in asymptomatic children and adults (Fig. 4). SARS-CoV-2 RNA loads in asymptomatic children (median 6.20 log₁₀ copies/mL) and adults (6.48 log₁₀ copies/mL) were comparable in magnitude (p 0.97). Moreover, no correlation was found between age and SARS-CoV-2 load (ρ 0.066, p 0.44 for children and ρ 0.020, p 0.62 for adults) (Fig. 3C and D).

Comparison of URT SARS-CoV-2 RNA load in symptomatic versus asymptomatic children and adults

Children with COVID-19 symptoms displayed slightly higher SARS-CoV-2 RNA load than their asymptomatic counterparts (Fig. 5A), although statistical significance was not reached (p 0.61). In adults, median estimated SARS-CoV-2 RNA load was significantly

higher in symptomatic than asymptomatic subjects (p < 0.001) (Fig. 5B).

Inference of the percentage of children and adults presumably shedding infectious virions

We previously reported that SARS-CoV-2 could not be cultured from NP specimens returning $C_T > 25$ (<5.9 log₁₀ copies/mL) by the TaqPath COVID-19 RT-PCR [16]. We investigated the distribution of specimens yielding $C_T < 25$ across children and adults. The data are shown in [Supplementary Material Fig. S1](#). Overall, the percentage of NP specimens returning SARS-CoV-2 N RT-PCR C_T s below the abovementioned threshold was similar for symptomatic children and adults (p 0.28) and was also comparable between asymptomatic children and adults (p 0.87). Among children, that percentage was higher for those aged under 3 years, although the difference was not statistically significant (p 0.22). For most age groups the percentage was higher in symptomatic subjects than in asymptomatic ones, although these differences did not reach statistical significance (p > 0.5).

Assessment of the cellularity of NP specimens collected from pediatric and adult participants

To assess the quality of NP specimens collected from children and adults regarding cellularity, we randomly selected 30 samples from each population group ($n = 60$) that were matched in SARS-

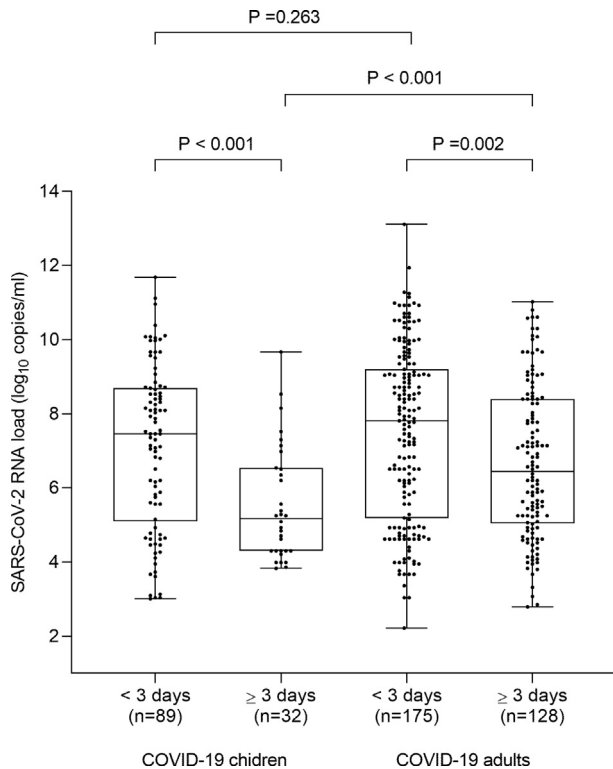


Fig. 2. Initial severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA loads in nasopharyngeal specimens from children and adults with coronavirus disease 2019 (COVID-19) according to the time of sampling after symptom onset. Medians are indicated by midlines; the top and bottom edges of boxes represent the interquartile range (IQR). Whiskers indicate the upper and lower values. The number of patients in each group as well as p values for comparisons between groups (median SARS-CoV-2 RNA levels) are shown.

CoV-2 RNA load (median 5.70 log₁₀ copies/mL, range 3.5–11.6 log₁₀ copies/mL in specimens from children; median 6.60 log₁₀ copies/mL, range 2.2–10.9 log₁₀ copies/mL in specimens from adults; p 0.99). These specimens were assayed with an in-house-designed RT-PCR amplifying the housekeeping GUSB gene. The C_T of NP samples obtained from children and adults did not differ significantly (median C_T 28.1, range 24.8–32.7, and median C_T 29.0, range 25.2–31.7, respectively, p 0.3), suggesting that SARS-CoV-2 RNA loads measured in the two population groups were not biased by differences in cellularity across NP specimens.

Discussion

Several major findings arose from the current study. First, overall there was no significant difference in URT SARS-CoV-2 RNA load at the time of presentation between COVID-19 paediatric and adult patients. Furthermore, the percentage of NP specimens potentially yielding infectious virions, as previously estimated [16], was similar across children and adults. Interestingly, a subanalysis categorizing patients by time to specimen collection from symptom onset revealed that SARS-CoV-2 RNA loads in children and adults were comparable at early times (within 2 days), when peak levels are likely to be reached [19], but were significantly lower in children after day 2, suggesting a faster URT SARS-CoV-2 RNA clearance rate in children. In accordance with our data, Baggio et al. [11] found similar estimated SARS-CoV-2 viral loads in children and adults sampled within the first 5 days after onset of symptoms. Likewise, Heald-Sargent et al. [9] found that preschool- and school-aged children sampled within 1 week after symptom onset display

SARS-CoV-2 RNA loads similar to those of their adult counterparts. In contrast, a slightly lower SARS-CoV-2 RNA load in children than in adults was reported in a German study [8]; however, information on symptom onset was not provided [8].

Consistent epidemiological evidence suggesting a less significant role of children as main drivers of SARS-CoV-2 spread in the community, as compared to adults, has been published [20–23]. Our data do not necessarily challenge this assumption, as onward transmission may not be associated only with the magnitude of SARS-CoV-2 RNA load in the URT [2–5,10,13], but also with the nature of symptoms (cough is less likely to develop in children compared to adults), lung capacity, and the ability to release aerosols—which seems to be lower in children than in adults, in particular in the absence of symptoms [24]—as well as differential social contacts and mixing patterns. In fact, a recent Danish study suggested a stronger correlation between age and transmission risk than between the C_T value and transmissibility [25]. Furthermore, our finding that SARS-CoV-2 clearance in the URT may proceed at a faster rate in children compared to adults gives further support to this argument.

Second, pairwise comparison analyses revealed no significant differences in SARS-CoV-2 RNA load across age groups, in either symptomatic children or adults; in children, a similar conclusion can be derived from the study by Kociolek and colleagues [12]. In contrast, age-related differences in SARS-CoV-2 RNA load have been reported previously in children [9,10]; specifically, young children (<5 years old) had significantly lower median SARS-CoV-2 RT-PCR C_T values than older children and adults. Differences in sampling times across these studies may account for the discrepancy.

Third, SARS-CoV-2 transmission to susceptible individuals from asymptomatic infected adults has been documented and postulated to facilitate virus dissemination in the community [26,27]. In fact, it has been estimated that at least 50% of new SARS-CoV-2 infections may originate from exposure to individuals with infection but without symptoms (asymptomatic or pre-symptomatic) [28]. Yet, data from several studies, including two systematic reviews [29–32], clearly suggest that asymptomatic patients are responsible for fewer secondary infections than individuals with symptoms. Here, in contrast to data reported in previous studies [33,34], we observed higher viral loads in symptomatic than in asymptomatic adults; of note, a wide range of SARS-CoV-2 RNA loads were detected in both asymptomatic children and adults, likely reflecting the broad spectrum of NP collection times after exposure to the presumed index case which, it should be noted, was not dissimilar between children and adults (a median of 7 days, range 1–10 days after diagnosis of the presumed index case for both study groups). Our observation is compatible with the above epidemiological data, given the known link between the magnitude of SARS-CoV-2 RNA load in the URT and the level of contagiousness [2–5]. Regarding children, we found nevertheless rather comparable SARS-CoV-2 loads in symptomatic and asymptomatic individuals, although a subtle trend towards higher viral loads was seen in the former. Although speculative, dating of symptom onset could have been more inaccurate in children than in adults, resulting in delayed testing, well beyond the time at which SARS-CoV-2 RNA peak load may have been reached. Our data concur with those of Hurst et al. [35], but contradict those of Kociolek et al. [12] which clearly pointed to lower SARS-CoV-2 RNA loads in asymptomatic children than in those with mild to moderate COVID-19. In this regard, it must be stressed that in our study asymptomatic individuals were tested relatively soon after exposure (median 7 days), whereas in Kociolek's study the authors admit a potential population bias towards lower SARS-CoV-2 loads due to an excessive number of remote infections (>10 days) detected via screening programmes (i.e. hospital pre-admission).

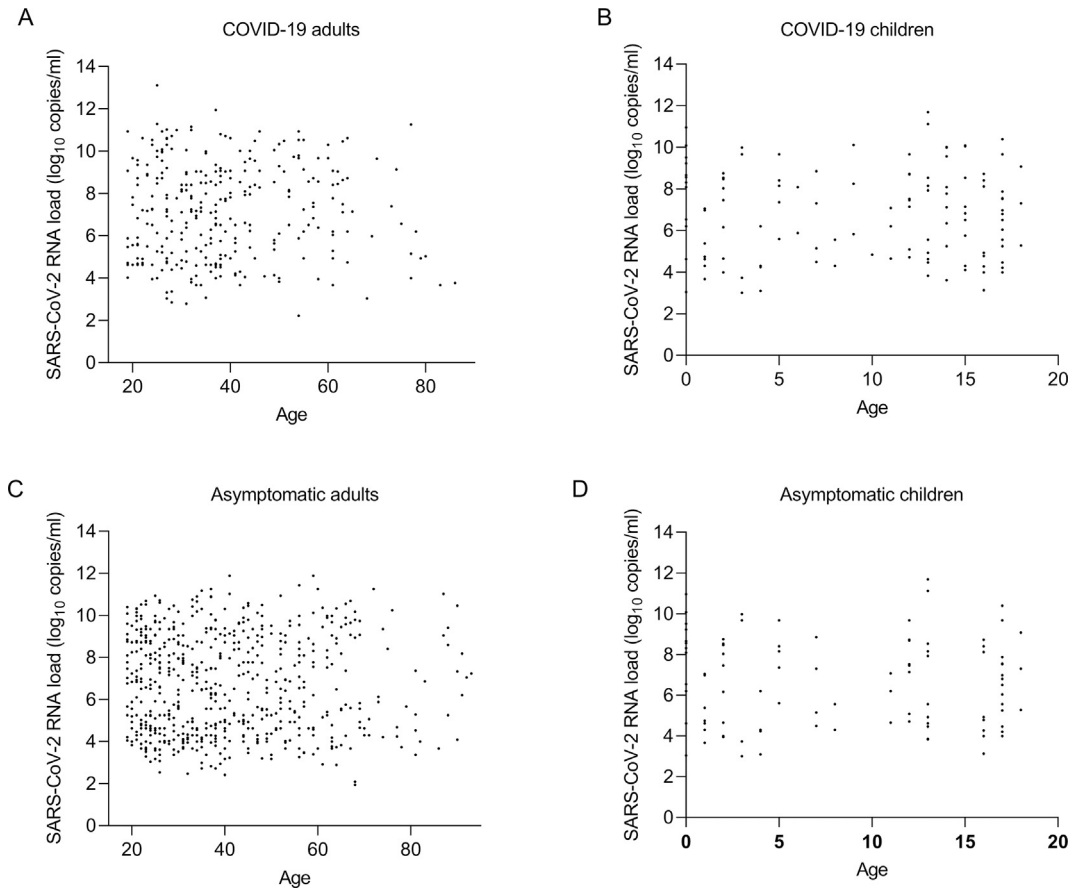


Fig. 3. Correlation between initial severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA load in nasopharyngeal specimens from (A) adults and (B) children with coronavirus disease 2019 (COVID-19), and from (C) asymptomatic adults and (D) children and age of participants.

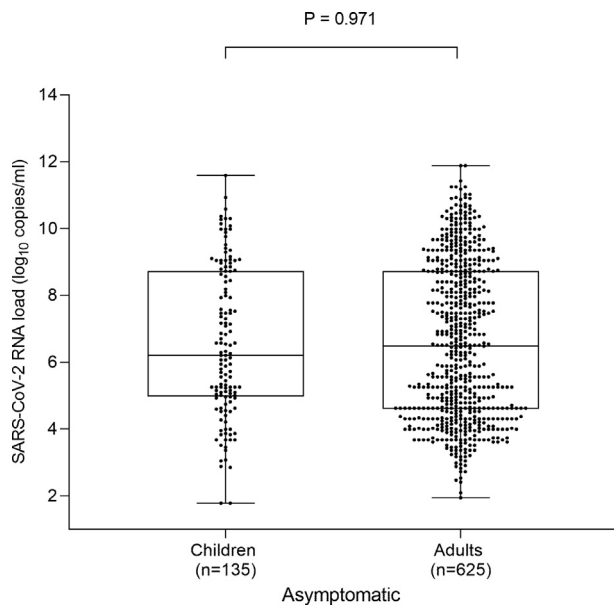


Fig. 4. Overall initial severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA loads in nasopharyngeal specimens from asymptomatic children and adults with coronavirus disease 2019 (COVID-19). Medians are indicated by midlines; the top and bottom edges of boxes represent the interquartile range (IQR). Whiskers indicate the upper and lower values. The number of patients in each group as well as p values for comparisons between groups (median SARS-CoV-2 RNA levels) are shown.

Like the majority of commercially available SARS-CoV-2 RT-PCRs, the RT-PCR assays used in the current study do not co-amplify a housekeeping gene, thus precluding assessment of sample cellularity. Given the widely varying quality of NP specimens [18], which impacts significantly on estimated SARS-CoV-2 RNA loads [36], we compared a randomly selected set of NP specimens from children and adults for their cellular content using a housekeeping-gene RT-PCR set in parallel. We found overlapping C_T s in samples from both subject groups, making it unlikely that differences in cellularity had a major impact on our results. However, only a small number of NP specimens was screened for their cellular content.

The current study has several limitations. First, dissimilarities in the timing of NP collection across symptomatic and asymptomatic individuals may have blurred true differences in SARS-CoV-2 RNA load across groups. While the kinetics of SARS-CoV-2 RNA load in URT has been clearly established in symptomatic individuals, with viral load peaking around the time of symptom onset [19], it remains to be precisely characterized in asymptomatic subjects. In fact, a population bias of this cohort towards higher viral loads in the asymptomatic population group in the current study cannot be ruled out. Second, participants were categorized as asymptomatic if they reported to be free of symptoms at the time of positive RT-PCR testing. We had no data as to their clinical outcome, that is, whether they were presymptomatic at the time of sampling or developed symptoms afterwards. Third, only initial SARS-CoV-2 loads were taken into consideration in the analyses, so that we could not have captured the true virus replication rate on an individual basis. Fourth, no attempt was made to subcategorize individuals according to their baseline medical condition.

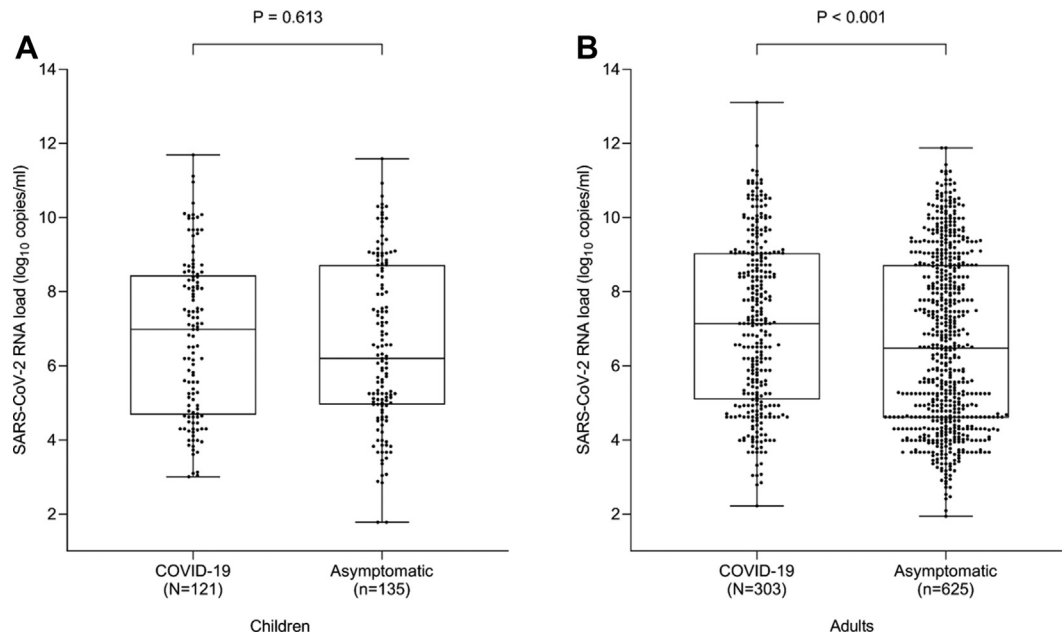


Fig. 5. Comparison of initial severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA loads in nasopharyngeal specimens from (A) children and (B) adults either asymptomatic or presenting with coronavirus disease 2019 (COVID-19). Medians are indicated by midlines; the top and bottom edges of boxes represent the interquartile range (IQR). Whiskers indicate the upper and lower values. The number of patients in each group as well as p values for comparisons between groups (median SARS-CoV-2 RNA levels) are shown.

Conclusion

In summary, URT SARS-CoV-2 RNA loads in non-hospitalized COVID-19 or asymptomatic COVID-19 children of all ages were not significantly different from viral loads seen in adults; however, URT SARS-CoV-2 shedding may be shorter in children than in adults. Nevertheless, it is important to note that onward transmission is not only associated with the magnitude of SARS-CoV-2 RNA load in the URT.

Author contributions

RC, FB, EA, IT, DS, CP and JC: methodology and data collection. RC, FB: formal analysis. RC, FB, CM-C and DN: conceptualization and validation of data. SC-S, AB-F, MILC, JRB-M and CM-C were physicians in charge of children. DN: writing the original draft. All authors reviewed and approved the final draft.

Transparency declaration

The authors declare no conflicts of interest. This work received no public or private funds. EA holds a Juan Rodés research contract (JR18/00053) from the ISCIII (Carlos III Health Institute, *Instituto de Salud Carlos III* in the original Spanish). IT holds a Río Hortega research contract from the Carlos III Health Institute (Ref. CM18/00221).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cmi.2021.08.001>.

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