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Abbreviations: ACE angiotensin-converting enzyme; CLIA chemilluminescence immunoassay; COVID-19 Coronavirus disease 2019; CT cycle threshold; GSFHC Germano Sinval Faria Health Center; IgG immunoglobulin G; GI gastrointestinal; GIS Geographic Information System; IQR Interquartile Range; LMICs Low Middle Income Countries; MIS-C Multisystem Inflammatory Syndrome in Children; PY person years; REDCap Research Electronic Data Capture, RT-PCR Reverse Transcription Polymerase Chain Reaction; SARS-CoV-2 Severe Acute Respiratory Syndrome Coronavirus 2; and VL viral load

Article Summary
We investigated the role of children in household SARS-CoV-2 transmission. Pediatric infections may follow exposure to adolescent or adult household contacts with recent infection.
What’s Known on This Subject
Studies have reported that children and adolescents who attend summer camp and social events away from home can introduce the virus to their household contacts.

What This Study Adds
Children do not seem to be the source of infection within their households and most frequently acquire SARS-CoV-2 from adults.

Contributors’ Statement
Dr. Lugon and Dr. Brasil conducted a literature search, designed the data collection instruments, collected and interpreted data, and wrote the initial and final drafts of the manuscript. Professors Fuller and Nielsen-Saines contributed to the literature search, data interpretation, and contributed to the writing of the initial and final drafts of the manuscript.

Ms. Damasceno, Ms. Fernandes, Ms. Salgado, and Dr. Abreu designed the data collection instruments, collected data, and reviewed and revised the manuscript.

Dr. Calvet, Dr. Resende, Dr. Matos, Dr. Machado, Mr. Malta, Dr. Cruz, Dr. Bastos, Dr. Guaraldo, Professor Whitworth, Professor Smith, Dr. Siqueira, and Dr. Carvalho contributed to data analysis, reviewed the manuscript for important intellectual content.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.
ABSTRACT

Objective: To investigate the dynamics of SARS-CoV-2 infection in a vulnerable population of children and their household contacts.

Methods: SARS-CoV-2 RT-PCR assays and COVID IgG serologies were performed in children and their household contacts following enrollment during primary healthcare clinic visits. Participants were followed prospectively with subsequent specimens collected through household visits in Manguinhos, an impoverished urban slum (favela) in Rio de Janeiro at 1, 2, 4 weeks and quarterly post study enrollment.

Results: 667 participants from 259 households were enrolled from May to September 2020. This included 323 children (0 - 13 years), 54 adolescents (14 - 19 years) and 290 adults. Forty-five (13.9%) children were SARS-CoV-2 PCR+. SARS-CoV-2 infection was most frequent in children < 1 year (25%) and 11-13 year old children (21%). No child had severe COVID-19 symptoms. Asymptomatic infection was more prevalent in children < 14 years of age than in those ≥14 years (74.3% and 51.1%, respectively). All children (N=45) diagnosed with SARS-CoV-2 infection had an adult contact with evidence of recent infection.

Conclusions: In our setting, children do not seem to be the source of SARS CoV-2 infection and most frequently acquire the virus from adults. Our findings suggest that in settings such as ours, schools and childcare potentially may be reopened safely if adequate COVID-19 mitigation measures are in place and staff are appropriately immunized.
INTRODUCTION

As of March 2021, more than 114 million confirmed cases of SARS-CoV-2 have been reported worldwide, 10% of which occurred in Brazil. Since the first report of COVID-19 in Brazil on February 26, 2020, more than 10.6 million confirmed cases and 256,000 deaths have been reported in the country, which is second only to the US in the absolute number of deaths due to COVID-191. Rio de Janeiro has the highest number of COVID-19 deaths of any city in Brazil2. Because children are generally pauci-symptomatic and tend to adhere less to hygiene and social distancing practices, they could potentially be a significantly underappreciated source of SARS-CoV-2 transmission. The concern about asymptomatic shedding of SARS-CoV-2 by children has also motivated many countries to close schools as one of the tools to halt the spread of infection3.

Studies involving family nuclei are an attractive means of investigating transmission of acute respiratory infections as well as clinical evolution of disease in individuals of different ages. The high frequency and intensity of contact between family members generates an environment conducive to transmission, particularly in dense urban households. Some studies have investigated families to analyze the transmission of influenza4-6 but few have explored SARS-CoV-2 infection and transmission7.

A better understanding of the role of children in transmission dynamics is of paramount importance for the development of guidelines for safely reopening schools8 and other public spaces and also for the development of immunization guidelines for pediatric populations. This is particularly salient in low-socioeconomic status communities such as slums, where household density is high. Like other LMICs, multigenerational dwellings are common in Brazil9,
potentially providing opportunities for transmission between pediatric population and older age groups. The main objective of this study was to investigate the dynamics of SARS-CoV-2 infection in children and their household contacts living in a vulnerable urban slum in Rio de Janeiro.

METHODS

We recruited and followed children less than 14 years of age who visited a primary healthcare facility for any reason (i.e., immunization, routine consultation, emergency care, accompanying relatives to the clinic, etc.) and additional children who shared the same residential address. Recruitment took place at the Germano Sinval Faria Health Center (GSFHC), a primary healthcare center located in the Northern sector of the city of Rio de Janeiro. GSFHC serves in average 21,000 children and adults per month, providing primary care and immunization services to the community of Manguinhos, an impoverished urban slum (favela) which lacks public services such as sanitation and electricity. Residents are assigned to receive care at this clinic based on their address in accordance with the policies of Brazil’s public healthcare system. Manguinhos is traversed by two rivers and an aqueduct approximately 5 km long, referred to hereafter as “canals”. The lowest-income households of the community are located along the canals, which are polluted with trash and sewage.

Following enrollment, children and their household contacts received home visits from study personnel. Home visits occurred 1, 2, and 4 weeks after enrollment, then quarterly. SARS-CoV-2 PCR assays and IgG serology were performed on all children and their household contacts, following written informed consent by the parent or legal guardian or their own informed
consent if \( \geq 18 \) years of age. Children age 6 years or older also provided written assent to study participation. Seroprevalence was defined as a positive serologic result which was counted once for each positive individual, on the date of their first positive result. Nasopharyngeal swabs were tested by real time RT-PCR to amplify the E gene and the RdRp region of the Orf1ab gene of SARS-CoV 2 (Charité/Berlin, Germany). Cycle thresholds (CT) less than 40 were classified as positive. Rectal swabs were self-collected in the case of adults, collected by the study pediatrician for children under 2 years of age, and by the mother for children older than 2 years. Rectal swabs were tested by real time RT-PCR using two sets of primers/probes targeting the virus nucleocapsid N gene (N1 and N2 region). SARS-CoV-2 serology (IgG) was performed using a chemilluminescence immunoassay (CLIA) targeting the N gene (Abbott Laboratories, Abbott Park, IL, USA). All assays were performed according to the manufacturer’s instructions.

Adolescent and adult household contacts were tested for SARS-CoV-2 by RT-PCR, independent of the presence of symptoms. We classified a participant as positive if at least one of their serial samples collected during the study tested positive. In addition, children and household contacts completed a study questionnaire which collected socio-demographic variables including number of residents and number of rooms; and the proportion of children living with siblings, grandparents, and other family members. Targeted physical exams of all children and symptomatic adults were performed concurrently. All data and laboratory results were recorded through a Research Electronic Data Capture tool - REDCap (Vanderbilt University; Nashville, TN). Household size was defined as the number of residents per room.
The primary outcome was the frequency of SARS-CoV-2 infections (positive RT-PCR and IgG antibodies) identified in the study population. We measured infection dynamics by assessing the percent of children < 14 years of age who were PCR-positive for SARS-CoV-2 and concurrently had an adult contact with positive SARS-CoV-2 IgG antibodies, or history of past COVID-19. This was defined as the presence of a recent respiratory illness accompanied by anosmia or ageusia. We inferred that if SARS-CoV-2 transmission were primarily from adults and adolescents to children, PCR positive children would have an adult or adolescent contact who had positive IgG antibodies to SARS-CoV-2, or a clinical history suggestive of past COVID-19. In addition, we assessed the timing of peak SARS-CoV-2 IgG prevalence in children vs. adults and adolescents. We hypothesized that if transmission were primarily from adults and adolescents to children, the peak IgG prevalence in adults and adolescents would occur before peak IgG prevalence in children.

Prior to recruitment the necessary sample size was determined. We calculated the sample size required to estimate the prevalence of SARS-CoV-2 in children as

\[
n = \frac{NZ^2p(1-p)}{d^2(N-1)+Z^2p(1-p)}
\]

where \(n\) is the number of children that must be sampled to estimate the prevalence of SARS-CoV-2 with 95% confidence. \(Z\) is the critical value of the standard normal distribution corresponding to this confidence level. We defined \(d\), the allowable margin of error, as 5%. \(N\) is defined as the number of children treated at GSFHC. We obtained the number of children < 14 years of age treated at the clinic in January, February, and March 2020\(^{13}\). We defined \(N\) as 4040, which is the mean number of children treated over this three month period. \(p\) is defined as an initial estimate as to the prevalence of SARS-CoV-2. New York City has a population density of 10,000 residents/km\(^2\) and SARS-CoV-2 seroprevalence of approximately 30%\(^{14}\). As Manguinhos has a
similar population density\textsuperscript{15}, we defined $p$ as 30%. Using these definitions, to estimate the incidence of SARS-CoV-2 with 95% confidence and allowing for 5% loss to follow-up, we calculated a sample size of 314 children was required. All tests were performed with Stata/IC 16.1 and SAS 9.4. Boschloo’s test was used to compare the rate of positivity by PCR between spans of three weeks within the study period.

A geographic information system (GIS) was created by assigning households to one of nine neighborhoods within the slum based on the participant’s home address. For each neighborhood, we calculated the length of roads as a proxy for access to public transportation, the length of open canals as a proxy for sanitation, and distance from GSFC. All calculations were performed in ArcGIS Desktop 10.6.1. We calculated Spearman’s correlation between these variables and the percentage of children from the neighborhood who tested positive for SARS-CoV-2 by RT-PCR.

The study was approved by the Brazilian National Ethics Committee (CONEP) under register number 30639420.0.0000.5262.

**RESULTS**

Recruitment and follow-up took place between May 18 and September 24, 2020. Fully 78.6 % of individuals approached consented to study participation. We had 20.5% refusals within the pediatric age group. We enrolled 323 children less than 14 years of age, 54 adolescents aged 14-19 years, and 290 adults older than 19 years who were household contacts of participating children (Fig. 1). A total of 259 households were studied. The median number of persons per household was 4 (IQR: 3-5).
Among 323 children, 41 were positive for SARS-CoV-2 by RT-PCR in their first visit and 4 in the second visit, totaling 45 positive children identified during the study period (13.93%). Among children, the incidence of new cases identified by RT-PCR was higher in May than in July ($p=0.022$), and rose again from July to September (Fig. 2), though the increase was not statistically significant ($p=0.13$). This is similar to the temporal pattern of SARS-CoV-2 incidence in the city of Rio de Janeiro as a whole (Fig. 3)\textsuperscript{16}. With respect to SARS-CoV-2 antibody prevalence in the population, SARS-CoV-2 IgG seroprevalence declined from July to September in children and adults (Fig. 2).

Among 342 contacts aged 14 years or older, 13.16% were infected with SARS-CoV-2 as measured by RT-PCR. The rate of SARS-CoV-2 infection was higher in children < 1 year of age (25%) and pre-adolescents aged 11-13 years (21%) (Fig 4A). The frequency of SARS CoV-2 infection was also higher in the same age group when IgG results were considered (Fig 4B). Eight participants in our study had persistently positive results for SARS-CoV-2 RNA for more than 14 days: four symptomatic adults and four children, three of whom were symptomatic. Three persistently positive individuals had positive gastrointestinal (GI) and respiratory tract specimens. One additional persistently positive individual had positive GI specimens.

A total of 32.6% (79/242) of children under 14 years of age and 31.2% (72/231) of household contacts were IgG positive, indicating that they had already been exposed to SARS-CoV-2 by September 2020 (Fig. 4). Of the 45 children who were PCR-positive, 26 had an adult contact who provided specimens for SARS-CoV-2 testing at the time of pediatric study enrollment. All 26 samples from concurrent adult household contacts tested positive either by PCR or CLIA. The
19 remaining adult contacts did not consent to provide a sample of their own. However, all 19 adults reported with symptoms of suspected COVID-19.

The number of persons per room was not significantly correlated with the percentage of household members positive for SARS-CoV-2 by PCR or serology ($r=0.06, p > 0.05$) (Fig. S1). The proportion of children who lived with grandparent(s) (i.e., multigenerational family households) was not significantly different among those who were positive for SARS-CoV-2 by PCR versus those who were negative ($p=0.13$). Furthermore, the proportion of children who lived with no siblings vs. those with one or more siblings did not differ significantly between children who were PCR positive or PCR negative ($p=0.15$). No severe cases of COVID-19 were noted among these children and their household contacts including siblings. Differences in CT values among infected children were not significantly different, nor were differences in CT values by sample type, age, or household size (Figs. S2-4).

A total of 39 in 45 PCR positive children (87%) and 286 of 323 (89%) both positive and negative children could be assigned to a neighborhood based on home addresses. The percentage of children who tested positive for SARS-CoV-2 by RT-PCR per neighborhood varied from 0 to 33%. SARS CoV-2 prevalence was highest in the neighborhoods of Higienópolis and Vila Turismo, in northwestern Manguinhos and also in the neighborhood of Mandela in southeastern Manguinhos (Fig. 5). There was no significant correlation between percent positivity and road length ($p=0.64$) or canal length ($p=0.59$), or proximity to the health clinic ($p=0.74$).
DISCUSSION

In our study, all children with SARS CoV-2 infection identified by RT-PCR had an adult or teenage household contact with suspected or confirmed SARS-CoV-2 before the child’s diagnosis. Unless these children were long term SARS-CoV-2 shedders, our results are compatible with the hypothesis that children were infected after or concurrent to household contacts, mostly their parents. To this extent, our preliminary results suggest that children do not seem to be the source of infection and most frequently acquire SARS-CoV-2 from adults, rather than transmitting it to them, which is different from previous studies.\textsuperscript{17, 18} A possible explanation for our findings would be that children are pauci-symptomatic in most cases and, therefore, would disperse fewer droplets and aerosols in the environment. The risk that children could infect others depends on factors such as SARS-CoV-2 viral load (VL) in the nasal secretions and feces, which could vary by age. However, comparisons of SARS-CoV-2 VL in younger and older children have yielded conflicting results, with one study reporting higher VL in the former\textsuperscript{19}, while others have found no effect of age\textsuperscript{20, 21}. In our population we observed that children under 1 year of age, and young teens tended to have the highest rates of infection and symptomatic disease. The former may be due to the close contact between children under 1 year and their mothers and the latter due to lower adherence to social distancing by young teens.

Adults might be more important spreaders because they have continued to work outside the home, while schools were closed early in the course of the pandemic, and remained closed through the study. Indeed, these adults were more vulnerable to SARS-CoV-2 because they maintained their activities as frontline workers, being exposed to a large number of individuals throughout the pandemic. Though schools remained closed, in mid-August 2020 other social
distancing rules were eased in Rio de Janeiro, most businesses reopened, and crowded public transportation resumed (Figs. 3, S5). Such reopening increased exposure among adults and increased their chance of being the real “vectors” of SARS CoV-2 infection as shown in the second wave of infections (Fig 2).

In the present study, we observed a higher proportion of infected children under one year of age as compared to other pediatric age groups, which may be attributable to direct contact with their mothers. A study in China reported a SARS-CoV-2 infection prevalence of 17% in children less than a year of age\textsuperscript{22}, whereas in our study the prevalence was approximately 30%. Ours is one of only a handful of studies to date which investigated SARS-CoV-2 infection in non-hospitalized children, all of whom resided in the same community. Due to factors such as the risk of urban violence in Manguinhos, children do not play outside alone until they are usually over 5 years of age. On the other hand, there is considerable variation among 5 to 12 year old children regarding the time spent outside of the home. Children 13 years of age or older typically spend a substantial proportion of time outside their home.

Approximately one-third of household contacts in our study were IgG positive, indicating that they had already been exposed to SARS-CoV-2 by August 2020, a higher prevalence of infection than that reported for the general population of Rio de Janeiro during that time period (i.e., 7.5% [4.2-12.2] versus 33% [28.6-37.6])\textsuperscript{23}. Although the duration of protection after infection remains unknown, in most immunization protocols, seropositive individuals are eligible to vaccinated as this remains the principal manner of reducing severe forms of COVID. It is also hoped that immunization will reduce transmission. These results are important for planning the reopening of
both schools and daycare centers, which have remained closed since March and also for the development of COVID-19 immunization strategies, such as prioritizing vaccination of teachers and childcare professionals.

Our results underscore the importance of including children in vaccine trails. Furthermore, if adults are immunized and children are not, children may continue to perpetuate the epidemic, highlighting the difference between vaccination strategies based on individual protection as opposed to vaccination strategies aimed at achieving herd immunity. If minimally 85% of susceptible individuals need to be immunized to curb the COVID-19 pandemic in high incidence countries, this level of protection can only be achieved with the inclusion of children in immunization programs, especially in Brazil, where 25% of the population is under 18 years old.

Study limitations included the logistical challenges of conducting home visits in this community, due to the risk of violence towards study staff, resulting in variable adherence to study procedures, missed visits, and delays in the enrollment of families. The relaxation of social distancing measures also made it more difficult to recruit adults, especially males, who returned to work and thus became unavailable. We might have underestimated the number of past infections if we tested too soon after infection onset or too late following infection because of waning of antibodies. This was difficult to determine because the date of infection was largely unknown in children and household contacts, most of whom were asymptomatic. Furthermore, the absence of IgG antibodies could be explained by effective innate immunity leading to robust
cellular immune responses after exposure to SARS-CoV-2 with no subsequent antibody immune responses

CONCLUSION

In summary, children do not seem to be the source of SARS-CoV-2 infection in our setting. Our findings demonstrate that most frequently children acquire infection from adults, rather than transmitting it to them. Our results support the strategy of safely reopening schools and day-care centers in our setting, particularly with strict COVID-19 mitigation measures and staff immunization. In low-resource settings such as ours, this is critical because access to online classes remains very limited.

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Figure 1. Flowchart of children enrolled in the study.
Figure 2. A. Incidence of SARS-CoV-2 based on RT-PCR per 100 person years. B. Prevalence of SARS-CoV-2 IgG antibodies. *p < 0.05. “Tested” denotes number of children < 14 or individuals >= 14 in each three week period. “Pos” denotes number positive for SARS-CoV-2 by RT PCR.
Figure 3. Incidence of SARS-CoV-2 in the city of Rio de Janeiro, May 18 – September 28, 2020. Bars represent the confirmed number of cases based on the date of symptom onset. The dotted line is the 7-day moving average of cases (Fig. S5 shows the dates of non-pharmaceutical interventions to control the epidemic).
Figure 4. Frequency of SARS-CoV-2 positive tests by age group in Manguinhos.
Figure 5. Distribution of SARS-CoV-2 in Manguinhos by neighborhood. The size of each pie chart is proportional to number of children recruited from the neighborhood.
Supplementary material

Figure S1. Household size vs. CT-value in children. The CT-value of PCR positive children did not vary with household size defined as the number of persons per room ($r=-0.14, p=0.39$). The solid line is a linear regression.
Figure S2. CT-value of RT-PCR positive children < 14 years of age with and without symptoms. Although the median CT-values of children with symptomatic were lower than in those who were asymptomatic, the difference was not significant ($p=0.28$).
Figure S3. CT-value of PCR-positive children < 14 years of age by sample type. The median CT-value for naso-oral, saliva, and rectal samples was not significantly different ($p=0.53$).
Figure S4. CT-value of PCR-positive children < 14 by age. The median CT-value of children under five years of age was not significantly different from that of children 5-14 years of age ($p=0.54$).
**Figure S5.** Non-pharmaceutical interventions to control SARS-CoV-2 in the city of Rio de Janeiro, March-September, 2020.

- **March 2020:**
  - Start of social distancing and quarantine.
  - 3-27 School closure.
  - Closure of non-essential businesses.
  - Outpatient medical care suspended.

- **April 2020:**
  - Declaration of a state of disaster.
  - Mask use mandatory.

- **May 2020:**
  - Bars closed, parking along the beach prohibited, along with forming crowds in public, and non-essential private construction projects.
  - Churches reopen.

- **June 2020:**
  - Mayor appoints reopening committee.
  - Malls and stores reopen.
  - Bars and restaurants reopen.

- **August 2020:**
  - Business and scientific events permitted.

- **September 2020:**
  - The city completes the final phase of the Mayor’s reopening plan.

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**School closure**
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