Received: 15 May 2020 | Revised: 10 July 2020 | Accepted: 16 November 2020

DOI: 10.1002/jmv.26689

SHORT COMMUNICATION

JOURNAL OF MEDICAL VIROLOGY WILEY

No SARS-CoV-2 carriage observed in children attending daycare centers during the intial weeks of the epidemic in Belgium

Stefanie Desmet[1] | Esra Ekinci[2] | Ine Wouters[2] | Bram Decru[1] | Kurt Beuselinck[3] | Surbhi Malhotra-Kumar[4] | Heidi Theeten[2]

[1]KU Leuven Department of Microbiology, Immunology and Transplantation, Laboratory of Clinical Bacteriology and Mycology, Campus Gasthuisberg, Leuven, Belgium

[2]Centre for the Evaluation of Vaccination, Vaccine and Infectious Disease Institute, University of Antwerp, Campus Drie Eiken, Wilrijk, Belgium

[3]Department of Laboratory Medicine and National Reference Centre for Respiratory Pathogens, University Hospitals Leuven, Leuven, Belgium

[4]Laboratory for Medical Microbiology, Vaccine and Infectious Disease Institute, University of Antwerp, Campus Drie Eiken, Wilrijk, Belgium

Correspondence
Esra Ekinci, Centre for the Evaluation of Vaccination, Vaccine and Infectious Disease Institute, University of Antwerp, Campus Drie Eiken, Universiteitsplein 1, 2610 Wilrijk, Belgium.
Email: Esra.Ekinci@uantwerpen.be

Funding information
Fonds Wetenschappelijk Onderzoek, Grant/Award Numbers: 1150017N, 1523518N; KU Leuven

Abstract

To gain knowledge about the role of young children attending daycare in the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) epidemic, a random sample of children (n = 84) aged between 6 and 30 months attending daycare in Belgium was studied shortly after the start of the epidemic (February 29th) and before the lockdown (March 18th) by performing in-house SARS-CoV-2 real-time polymerase chain reaction. No asymptomatic carriage of SARS-CoV-2 was detected, whereas common cold symptoms were common (51.2%). Our study shows that in Belgium, there was no sign of early introduction into daycare centers at the moment children being not yet isolated at home, although the virus was clearly circulating. It is clear that more evidence is needed to understand the actual role of young children in the transmission of SARS-CoV-2 and their infection risk when attending daycare.

KEYWORDS
carriage, children, COVID-19, daycare, SARS-COV-2

1 | INTRODUCTION

In December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) had emerged in Wuhan, China, causing the 2019 novel coronavirus disease (COVID-19). This virus eventually had spread worldwide, and in March 2020, the World Health Organization announced SARS-CoV-2, a pandemic health emergency. Person-to-person transmission of SARS-CoV-2 takes place mainly through close contact with an infected person (mainly via respiratory droplets) and after touching infected objects.[1] Most common clinical manifestations of SARS-CoV-2 are fever, dry cough, shortness of breath, and respiratory failure and vary from very mild to severe symptoms.[2] Other symptoms in children are diarrhea, nausea/vomiting, fatigue, and abdominal pain.[3–5] Recently, in the United States, England, Italy, and France, rare cases with symptoms of Kawasaki-like multisystem inflammatory syndrome were reported.[6–9]

Current evidence indicates that older populations are most susceptible to severe presentations of COVID-19; however, there is less knowledge about disease severity and transmission among infants and children.[10] Evidence in China suggests that the clinical symptoms of COVID-19 may be less severe in children and thus harder to recognize.[11,12] Possible explanations could be: (1) children

might have fewer opportunities for exposure to pathogens or infected patients,[11] (2) lower expression of the angiotensin-converting enzyme II (ACE2) receptor as it is indicated that ACE2 is likely the receptor for SARS-CoV-2,[13] (3) children often get respiratory infections (including coronaviruses) in the winter and might therefore have higher antibody levels than adults,[11] (4) the immune system of children is still developing and may react differently on pathogens compared to adults' immune system.[14] The lower symptomatic disease incidence in the pediatric population raises the concern that this population could be an important source of SARS-CoV-2 transmission.[12]

Understanding the burden of carriage in children and the subsequent potential for transmission of severe acute respiratory syndrome-associated coronavirus (SARS-CoV-2), it is important to implement public health measures and to decide on the exit strategy for the current lockdown in Belgium. This study investigated the (asymptomatic) carriage of SARS-CoV-2 in children attending daycare centers (DCCs) during the period March 2–12, 2020, to get more insight into the possible role of children in the transmission of SARS-CoV-2 during the intial weeks of the SARS-CoV-2 epidemic in Belgium. Although the first confirmed case was on February 4th, 2020, the real start of the epidemic was detected from February 29th onwards, with a total of 689 confirmed cases spread over the whole country on March 13th.[15] Early March, Belgium had a massive introduction of travelers with COVID-19, which was just at the start of the Southern European epidemic. Contrary to the expectations and observations in other countries, Belgian case data and the results of a seroprevalence study (week March 30) underline the scattered spread of COVID-19 all over the country during the intial weeks of March.[16] Due to the restricted testing at that moment, no reliable Belgian incidence rates are available for March 2020, but seroprevalence of SARS-CoV-2 IgG was 2.9% at the end of March.[16]

2 | MATERIAL AND METHODS

2.1 | Ethical statement

The current study was in line with the Declaration of Helsinki, as revised in 2013. Approval to conduct the current study with ID 18/31/355 was obtained from the University of Antwerp and University Hospital of Antwerp ethics committee (Commissie voor Medische Ethiek van UZA/UA) on 29/07/2019. Informed consent allowed us to determine other respiratory pathogens.

2.2 | Study population and sampling

The current study was embedded in the nasopharyngeal (NP) carriage study that started in Belgium in 2016 to monitor changes in the proportions of pneumococcal serotypes in children between six and thirty months of age attending DCCs.[17] DCCs were randomly selected throughout Belgium. After written consent of at least one parent, a single NP swab (pediatric flocked swab (Copan®)) was collected. A questionnaire regarding the child's demographic and clinical characteristics, as well as pneumococcal vaccination status, was filled in by their parents. Signs of the common cold in children were defined as coughing and/or running nose and were registered at the moment of sampling.[17]

Sample collection during 2019–2020 was performed from the beginning of November 2019 to the end of March 2020. This collection period spanned the crucial first weeks of the COVID-19 epidemic in Belgium, thus enabling us to study the introduction, if any, of the SARS-CoV-2 virus in the daycare population. To determine the SARS-CoV-2 carriage, NP swabs taken from 84 children attending eight different DCCs spread over 6 of the 10 Belgian provinces (namely three in East Flanders, one in Antwerp, one in Brussels, one in Liège, one in Walloon Brabant, and one in Luxemburg) during the period of March 2–12, 2020 were analyzed. Of the 84 children included in this study, relevant population characteristics were: (1) 43 (52.4%) were girls; (2) at the moment of sampling, signs of common cold were observed in half of the children (51.2%); (3) just over half (56.1%) of the children had at least one sibling in the same household; (4) the majority of children (87.8%) stayed at least twice a week in daycare; and (5) the parents of the majority of the children (75.6%) were nonsmokers.

2.3 | In-house SARS-CoV-2 real-time polymerase chain reaction (PCR)

The collected swabs were transported on dry ice and stored in 1 ml STGG (skim milk, tryptone, glucose, glycerol). In-house SARS-CoV-2 real-time PCR targeting the E gene, making use of primers adapted from Corman et al.[18] was performed on 200 µl of the sample. The limit of detection of this PCR is 50 copies/ml and validation of this PCR indicated a 100% sensitivity taking results of Xpert Xpress SARS-CoV-2 and Hologic Aptima SARS-CoV-2 as the golden standard. Validation of the test on STGG medium was performed by adding 3 µl of COVID-19-positive sample ($n = 3$) to 300 µl STGG media, followed by storage in a refrigerator (4°C) for 24 h and afterward freezing at −80°C. C_t-values of the frozen STGG samples were similar to the C_t-values of the initial sample (1/100 diluted).

3 | RESULTS

All analyzed samples were negative for SARS-CoV-2, which means that shortly after the start of the epidemic (February 29th) and before the lockdown in Belgium (March 18th) no (asymptomatic) carriage of SARS-CoV-2 was detected in a random sample of children ($n = 84$) aged between 6 and 30 months attending daycare. Only one sample had an amplification curve (C_t value of 38.8). To confirm this weak signal, the extract of this sample was reanalyzed in triple and no amplification was observed. A limitation of our study is that we have no information on COVID-19-like symptoms in household members or caregivers.

4 | DISCUSSION

The result is in line though with other studies that suggest only a minimal role of children in the epidemic of SARS-CoV-2. Iceland reported children under 10 years of age less likely to be positive by reverse transcriptase-PCR testing than were persons 10 years of age or older in large-scale screening in a random sample of the general population (n = 13,080 of whom 848 children were all negative vs. 0.8% positives in the remaining sample).[19] Similarly, in Italy no children younger than 10 were infected in a large-scale survey before and after the start of the lockdown in the municipality where the first COVID-19 death in Italy was reported.[20] Among confirmed COVID-19 patients, the percentage of children, was as low as 1–5% in studies published up to March 18, 2020, and in the United States up to April 10.[3,21] Although children in China had a similar risk of infection as adults,[22] China reported a lower secondary attack rate to children than to adults (4% vs. 17%) in a household study.[23] Only a handful of deaths among children with COVID-19 have been reported worldwide, and generally, symptoms in children are mild.[24]

Limited evidence from contact research suggests that children less frequently infect other people than do adults, although similar viral loads as in adults were observed in an unpublished German study.[25] In the Netherlands, a household study showed that the spread of SARS-CoV-2 occurs mainly between people of approximately the same age, and that patients under 20 years of age had a smaller impact on the spread of the virus compared to adults.[26] A recent review found only 9.7% pediatric index cases among 31 intra-household transmission clusters identified from the literature.[27] These findings were supported by data from Guangzhou. They found even lower rate of children (around 5%) as index cases in households.[28] A case study of a cluster in France showed that there was no transmission from a COVID-19-positive child to any other person after exposure to more than a hundred children.[29] In Austria, 735 students and 128 staff were in close contact with nine children and nine adult school cases; here again, no transmission from the cases to other people was observed.[30]

Our study adds that in Belgium, where the epidemic was imported mainly by adult travelers, there was no sign of early introduction into DCCs at the moment children being not yet isolated at home, although the virus was introduced widespread, and immediately circulating according to case-based and seroprevalence data. It is clear that more evidence is needed to understand the actual role of young children in the transmission of SARS-CoV-2 and their infection risk when attending daycare.

ACKNOWLEDGMENTS

Heidi Theeten and Ine Wouters acknowledge Research Fund Flanders (1523518N and 1150017N) and Stefanie Desmet acknowledges KULeuven for funding.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

All authors contributed to study design and implementation. Esra Ekinci, Ine Wouters, and Heidi Theeten initiated, and Stefanie Desmet and Surbhi Malhotra-Kumar reviewed the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Esra Ekinci http://orcid.org/0000-0003-1101-5681

REFERENCES

1. Xu Y, Li X, Zhu B, et al. Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding. *Nat Med*. 2020;26(4):502-505.
2. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020; 579(7798):270-273.
3. Ludvigsson JF. Systematic review of COVID-19 in children shows milder cases and a better prognosis than adults. *Acta Paediatr*. 2020; 109:1088-1095.
4. de Souza TH, Nadal JA, Nogueira RJN, Pereira RM, Brandao MB. Clinical manifestations of children with COVID-19: a systematic review. *Pediatr Pulmonol*. 2020;55(8):1892–1899.
5. Cao Q, Chen YC, Chen CL, Chiu CH. SARS-CoV-2 infection in children: transmission dynamics and clinical characteristics. *J Formos Med Assoc*. 2020;119(3):670-673.
6. Jones VG, Mills M, Suarez D, et al. COVID-19 and Kawasaki disease: novel virus and novel case. *Hosp Pediatr*. 2020;10(6):537-540.
7. Riphagen S, Gomez X, Gonzalez-Martinez C, Wilkinson N, Theocharis P. Hyperinflammatory shock in children during COVID-19 pandemic. *Lancet*. 2020;395(10237):1607-1608.
8. Verdoni L, Mazza A, Gervasoni A, et al. An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. *Lancet*. 2020;395(10239): 1771-1778.
9. Toubiana J, Poirault C, Corsia A, et al. Kawasaki-like multisystem inflammatory syndrome in children during the covid-19 pandemic in Paris, France: prospective observational study. *BMJ*. 2020;369: m2094.
10. The Novel Coronavirus Pneumonia Emergency Response Epidemiology Team. The Epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19)—China, 2020. *China CDC Weekly*. 2020;2(8):113-122.
11. Dong Y, Mo X, Hu Y, et al. Epidemiology of COVID-19 among children in China. *Pediatrics*. 2020;145:e20200702.
12. Chen C, Cao M, Peng L, et al. Coronavirus disease-19 among children outside Wuhan, China (2/25/2020). *SSRN*. 2020.
13. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell*. 2020;181(2):271-280.
14. Abdulamir AS, Hafidh RR. The possible immunological pathways for the variable immunopathogenesis of COVID-19 infections among healthy adults, elderly and children. *Electron J Gen Med*. 2020;17(4): em202.
15. Sciensano. *COVID-19—Epidemiologische situatie op 14 maart 2020*. Sciensano; 14 March 2020.
16. Herzog S, De Bie J, Abrams S, et al. Seroprevalence of IgG antibodies against SARS coronavirus 2 in Belgium: a prospective cross-sectional study of residual samples. *medRxiv*. 2020. Preprint.

17. Wouters I, Desmet S, Van Heirstraeten L, et al. How nasopharyngeal pneumococcal carriage evolved during and after a PCV13-to-PCV10 vaccination programme switch in Belgium, 2016 to 2018. *Euro Surveill*. 2020;25(5):1900303.
18. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill*. 2020;25(3).
19. Gudbjartsson DF, Helgason A, Jonsson H, et al. Early spread of SARS-Cov-2 in the Icelandic population. *N Engl J Med*. 2020;383: 2184-2185.
20. Lavezzo E, Franchin E, Ciavarella C, et al. Suppression of COVID-19 outbreak in the municipality of Vo, Italy. *Nature*. 2020;584:425–429.
21. CDC. *Coronavirus disease 2019 in children—United States*. United States: Centers for Disease Control and Prevention (CDC); 2020.
22. Bi Q, Wu Y, Mei S, et al. Epidemiology and transmission of COVID-19 in Shenzhen China: analysis of 391 cases and 1,286 of their close contacts. *The Lancet*. 2020;20(8):911–919.
23. Li W, Zhang B, Lu J, et al. The characteristics of household transmission of COVID-19. *Clin Infect Dis*. 2020;71:1943-1946.
24. Castagnoli R, Votto M, Licari A, et al. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in children and adolescents: a systematic review. *JAMA Pediatr*. 2020;174:882.
25. Jones TC, Mühlemann B, Veith T, et al. An analysis of SARS-CoV-2 viral load by patient age. *medRxiv*. 2020.
26. RIVM. Kinderen en COVID-19. Rijksinstituut voor Volksgezondheid en Milieu (RIVM), Ministerie van Volksgezondheid, Welzijn en Sport. 2020. https://www.rivm.nl/coronavirus-covid-19/kinderen
27. Zhu Y, Bloxham CJ, Hulme KD, et al. Children are unlikely to have been the primary source of household SARS-CoV-2 infections. *medRxiv*. 2020.
28. Jing Q-L, Liu M-J, Yuan J, et al. Household secondary attack rate of COVID-19 and associated determinants. *The Lancet*. 2020;20: 1141–1150.
29. Danis K, Epaulard O, Bénet T, et al. Cluster of coronavirus disease 2019 (COVID-19) in the French Alps, February 2020. *Clin Infect Dis*. 2020;71:825-832.
30. National Centre for Immunisation Research and Surveillance (NCIRS). 2020. COVID-19 in schools—the experience in NSW. Australia: NCIRS.

How to cite this article: Desmet S, Ekinci E, Wouters I, et al. No SARS-CoV-2 carriage observed in children attending daycare centers during the intial weeks of the epidemic in Belgium. *J Med Virol*. 2021;93:1828–1831.
https://doi.org/10.1002/jmv.26689