Longitudinal testing for respiratory and gastrointestinal shedding of SARS-CoV-2 in day care centres in Hesse, Germany

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Summary
In a 12-week longitudinal study, neither respiratory nor gastrointestinal shedding of SARS-CoV-2 was observed in any of 859 children attending 50 day care centres in Hesse, Germany, during the pandemic. Only two out of 376 day care staff members tested positive in the weekly screening effort. The low positivity rate indicates that, with infection prevention measures in place, shedding of SARS-CoV-2 by asymptomatic children attending day care is uncommon in the context of low community spread.

Abstract

Background

With the pandemic of SARS-CoV-2 ongoing in Europe in June of 2020, day care centres were reopened in the state of Hesse, Germany, after the lockdown. The role young children play in the dynamics of the transmission was unknown.

Methods

We conducted a longitudinal study over a period of 12 weeks and two days (18th of June 2020 to 10th of September, 2020) to screen attendees and staff from day care centres in the state of Hesse, Germany, for both respiratory and gastrointestinal shedding of SARS-CoV-2. 859 children (age range 3 months to 8 years) and 376 staff members from 50 day care centres, which were chosen representatively from throughout the state, participated in the study. Parents were asked to perform both a buccal mucosa and an anal swab on their children once a week. Staff were asked to self-administer the swabs. RT-PCRs for SARS-CoV-2 were performed in a multiple-swab pooling protocol.

Results

7,366 buccal mucosa swabs and 5,907 anal swabs were analysed. No respiratory or gastrointestinal shedding of SARS-CoV-2 was detected in any of the children. Shedding of SARS-CoV-2 could be detected in two staff members from distinct day care centres. One was asymptomatic at the time of testing, and one was symptomatic and did not attend the facility on that day.

Conclusion

Detection of either respiratory or gastrointestinal shedding of SARS-CoV-2 RNA in children and staff members attending day care centres was rare in the context of limited community activity and with infection prevention measures in the facilities in place.

Introduction

In the pandemic of SARS coronavirus 2 (SARS-CoV-2), pre-school children are at a low risk of severe disease and death when infected with the virus.[1,2] When social distancing and closure of day care centres were applied to counter the first peak of cases of COVID-19 in Germany in March of 2020, young children were considerably affected by these measures. Restricting access to education is expected to have negative effects on the well-being of children of all age groups, with families of low socioeconomic status and families with children with special educational needs most likely to suffer.[3] Children are, indeed, believed to be main drivers of the transmission to the community of other respiratory viruses, such as influenza and rhinovirus.[4-6] But the role pre-school children play in transmission of SARS-CoV-2 was largely unknown when day care facilities reopened after the lockdown, with hygiene restricts in place,[7] on June 2nd of 2020, in the State of Hesse, Germany. Shedding light on the role children play in the dynamics of SARS-CoV-2 transmission ought to be a high priority. Findings from school-age children and adolescents cannot be readily transferred to younger children. Epidemiological data on the unique setting of day care centres are therefore needed.

Since children are often only mildly symptomatic or remain asymptomatic when infected with SARS-CoV-2, it is conceivable that they are shedding virus when attending day care centres, rendering rules that prevent symptomatic children from entering these facilities ineffective. While the viral load of infected children of all age groups was observed not to differ significantly from adults[8], it has not yet been conclusively demonstrated whether children are as likely as adults to transmit the virus to others or not. Chains of transmission among inapparently infected children could remain undetected without laboratory-based surveillance, and are hypothesized to thusly spill over to the community.[9,10]

To detect SARS-CoV-2 RNA for clinical and diagnosis and surveillance, nasopharyngeal (NP) or oropharyngeal swab specimens are commonly recommended. However, both of these sample collection methods need to be obtained by a health care professional using PPE (personal protective equipment) and may be very unpleasant or could cause injury to young children, prohibiting their use in longitudinal screenings. Alternative sample collections methods have been proposed to detect respiratory shedding, including saliva samples.[11] However, supplying these samples may be difficult for young children. Buccal mucosa swab samples can be easily and safely obtained from young children[12] by the caregivers, but testing sensitivity and the diagnostic window may be inferior to NP or throat swabs. Also examining stool or anal swab samples has previously been observed to increase both the diagnostic yield[13-16] as well as the diagnostic window.[17]

In the ***SAFE KiDS-Study*** (German: **SA**RS-CoV-2 **F**rüh**E**rkennung in **Ki**tas mit "**D**ual **S**wabs", English: Early Detection of SARS-CoV-2 in day care centres with "dual swabs"), we enrolled children and staff members from 50 day care centres chosen representatively throughout the State of Hesse, Germany, to reflect the distribution of the population between a densely population metropolitan region in the centre south, and more rural areas throughout the state. Participants were invited to be tested weekly for respiratory and gastrointestinal shedding by self-collected buccal mucosa and anal swabs for SARS-CoV-2 over a period of 12 weeks to determine whether viral shedding could be observed.

Measures to reduce the spread of SARS-CoV-2 in day care centers were put in place by the Hessian Ministry for Social Affairs and Integration and applied during the study period. These included barring children and staff with symptoms of COVID-19, other than a runny nose only, from entering the facilities, as well as denying access to individuals with known exposure to SARS-CoV-2. Access to the facilities was also denied to children if a household member was symptomatic, or was in quarantine due to contact with SARS-CoV-2. Wearing of masks was not mandatory for children, and only mandatory for staff when other adults were present. The access to the facilities was limited for parents and other adults.[7]

Methods

Study design

Hesse lies in the centre of Germany, and has 5,993,771 inhabitants, 5.2% (364,226) of whom are below the age of 6 years.[18] A representative sample of day care centres was selected by the State Office of Statistics of Hesse. The selected facilities were invited to participate in the study. 50 facilities were recruited (figure 1), which is about 1% of all day care centres in Hesse. 30 participants from each facility, preferably from one care group, compromising both children and staff members, were invited to provide self-collected swab samples once a week. Parents who participated in the study were asked to perform both a buccal mucosa swab as well as an anal swab ("dual swabs") from their children once a week before visiting the day care centre. They received written instructions and were provided access to a video, available with both English and German subtitles, explaining the goal of the study as well as the swabbing procedure. Written consent was obtained. Providing the swabs was voluntary each week, and parents were instructed not to force sample collection. Participating day care staff were instructed to self-administer the swabs. Samples were collected between 18th of June 2020 to 10th of September 2020. Only dry swabs were used. Samples were stored at room temperature and tested on the day of collection.

Laboratory testing

Testing for SARS-CoV-2 was performed at the Institute of Medical Virology, Goethe University Frankfurt, Germany. Before proceeding to reverse transcription polymerase chain reaction (RT-PCR) testing for SARS-CoV-2, samples were pooled in a 10-sample group-testing mini-pool protocol, that enables efficient use of reagents in a setting with a low pre-test probability, without significant loss in testing sensitivity.[19] In case of a negative result in the pooled sample, all individual samples receive a negative test result. When at least one of the two PCR targets (E-gene or ORF-region) was detected, all samples of the pool were individually tested. All RT-PCRs were performed on the Roche cobas® 6800 instrument (Roche diagnostics, Basel, Switzerland) according to manufacturer instructions. For all individual samples yielding a positive result for either one or both PCR targets, the public health authority was informed in accordance with the German Infection Protection Act.

To verify the pre-analytic quality of the samples, all of which were obtained without observation by a health-care professional, a sample of 800 buccal mucosa swabs were randomly selected from individual study participants to quantitatively test for GAPDH mRNA by RT-PCR. RNA was extracted with the QIAamp 96 Virus QIAcube HT Kit (QIAGEN, Hilden, Germany) and RT-PCR was performed with the Luna® Universal One-Step RT-qPCR Kit (New England Biolabs, Ipswich, Massachusetts) on the CFX96 Touch Real-Time PCR Detection System (BioRad, Hercules, CA, USA) according to manufacturer's instructions.

Questionnaires

At the end of the study, all study participants were asked to fill out a questionnaire to assess any exposure to SARS-CoV-2, and to evaluate whether an infection with SARS-CoV-2 had been diagnosed outside the study.

Statistical analysis

The study is analysed in a descriptive statistical assessment because of the low incidence rates. Rates are calculated together with 95% confidence intervals (CI).

Ethical approval

This study protocol was approved by the ethics board of the University Hospital Frankfurt, Goethe University Frankfurt am Main, Germany.

Role of the funding source

The *SAFE KiDS study* was commissioned by the Hessian Ministry of Social Affairs and Integration and was supported by Roche, Basel, Switzerland. The funder of the study did not contribute to study design, data collection, data analysis, data interpretation, or writing and submitting of the report for publication.

Results

Study participants and sample distribution

A total of 1,235 study participants from 50 day care centres were enrolled in the study. 859 (69.6%) of participants were children, the age range was 3 months to 8 years and 11 months (table 1). Most children were 4 or 5 years old. 376 participants (30.4%) were day care staff. The age range of staff members was 19 to 64 years. A total of 13,273 valid samples were tested (table 1). 7,366 (55.5%) of these samples were buccal mucosa swabs and 5,907 (44,5%) were anal swabs. 9,057 (68.2%) of samples were from the 859 children, of which 4,941 were buccal mucosa swabs and 4,116 were anal swabs. 4,216 (31.8%) of samples were from the 376 staff members, of which 2,425 were buccal mucosa swabs and 1,791 were anal swabs. The number of samples varied by week (figure 2) and was lower during summer recess, which was from calendar week 28 to 33. The median number of weeks that participants provided at least one sample was six for children and seven for staff members.

Results of testing for SARS-CoV-2 RT-PCR

Out of 7,366 buccal mucosa swab, SARS-CoV-2 RNA could not be detected in 7,364 (99.97%, 95% CI 99.90% to 100%). Both targets of SARS-CoV-2 RNA (ORF-region and E-gene) were detected in one sample, and only one target gene (E-gene) was detected in one sample. Out of 5,907 anal swab, SARS-CoV-2 RNA was not detected in 5,906 (99.98%, 95% CI 99.90% to 100%). In one anal swab sample, one out of two targets (E-gene) was detected.

Description of the detected cases

In calender week 26, SARS-CoV-2 RNA was detected in both the buccal mucosa swab (both PCR targets) and an anal swab (one target) of a day care centre staff member. She was asymptomatic and unaware of the infection at the time of testing. The infection was confirmed by independent testing. The day care center was ordered to be closed for quarantine by the local health department. The local 7 day-incidence of SARS-CoV-2 infections in the administrative distinct of the day care center was low, with 3.5 cases / 100,000 inhabitants, and the 7 day-incidence in children below the age of 8 years was 15.25 cases / 100,000 inhabitants.

In calendar week 34 of 2020, one PCR target (E-gene) was detected in the bucal mucosa swab of a staff member of another day care center. An anal swab was also provided and yielded a negative result. She was symptomatic at the day of testing, and self-isolated after dropping off the sample, and did not tend to children on that day. A positive result was obtained by independent RT-PCR testing. This day care center was also ordered to be closed for quarantine by the local health department. The local 7 day-incidence of SARS-CoV-2 infections was 35.95 cases / 100,000 inhabitants, and the 7 day-incidence in children below the age of 8 years was 26.18 cases / 100,000 inhabitants.

Assessment of pre-analytic validity of parent-collected and staff-self-collected buccal mucosa swab samples

800 buccal mucosa swab samples were selected and quantitatively tested for human GAPDH mRNA by RT-PCR, to determine whether material containing cells had been successfully collected. The sample represents 10.9% of all collected buccal mucosa swabs. GAPDH mRNA was detected in 715 (89.4%; 95% CI 87.0% to 91.4%) of these samples (figure 3).

Results from the questionnaires

557 questionnaires were sent in for participating children (return rate 64.8%), and 256 questionnaires were sent in from day care staff members (return rate 68.1%). The questionnaires were anonymously analysed. None of the participants reported to have been diagnosed with a SARS-CoV-2 infection (COVID-19) outside of the study during the study period. Table 2.

Community activity of SARS-CoV-2 in Hesse during the study

During the study period, the incidence of SARS-CoV-2 was relatively low, both for the over-all population as well as for children below the age of 6 years. Regional peaks in the 7-day-incidence in precincts with participating day care centers occurred with up to 66 cases / 100,000 inhabitants.

Discussion

To our knowledge, this is the first large study examining both respiratory and gastrointestinal shedding of SARS CoV-2 in a representative selection of day care centres during the pandemic. Because symptomatic children and staff were not allowed to attend the facilities during the study due to restricts in the pandemic, we assume that most study participants were asymptomatic at the time of sample collection.

The overall community incidence of SARS-CoV-2 in the study period was low, but varied by region and study week from 0 to 66 cases / 100,000 inhabitants. In this context, SARS-CoV-2 RNA was detected in none of 7,366 buccal mucosa and 5,907 anal swabs from a total of 859 children attending the day care centres. The absence of detectable respiratory or gastrointestinal shedding of SARS-CoV-2 in the self-collected swabs in any child that participated in our study is reassuring. The positivity rate of samples was low, even though measures recommended to contain transmissions in older children and adults, such as social distancing and wearing of masks, cannot always be applied in the care of young children.

The only two cases of shedding of SARS-CoV-2 that were detected in our study were in day care staff. Staff members appear to be the likely index case in clusters that were reported from ~~the~~ day care centres in recent reports from Poland the United States.[20,21] This is in accordance with a recent study that did not determine an increased risk for day care providers with exposure to child care

during the early pandemic in the USA.[27] While the number of cases detected in staff members in our study was too low to draw conclusions, it should be evaluated further whether screening of staff may be effective in preventing outbreaks of SARS-CoV-2 in day care centres. Our study indicates that screening asymptomatic, young children in a low incidence setting is likely to be ineffective in preventing outbreaks due to the extraordinarily low pre-test probability.

Despite the large number of tests, no single false positive result was observed, demonstrating that the RT-PCR is a highly specific test. Both cases that were detected in our study were later confirmed by independent evaluations. However, we cannot exclude that false negative results may have occurred. The buccal mucosa swab is less sensitive than a nasopharyngeal or throat swab[12], but could be performed by the caregivers in the study with acceptable pre-analytic quality of the samples without significant risk of injury to the child. The pre-analytic quality could be demonstrated by the large amount of buccal mucosa swabs that contained human cells, as determined by the RT-PCR of GAPDH mRNA. By also testing for SARS-CoV-2 in anal swabs we believe to have further reduced the risk of failing to detect children shedding the virus while attending the facilities. In a survey at the end of the study, none of the participants who returned the questionnaire reported to have received a positive test result for SARS-CoV-2 outside of the study (table 2).

This study was conducted in the summer of 2020, when activity of other respiratory pathogens was also low in Hesse, Germany, and children with symptoms of upper respiratory infection, other than runny nose only, were excluded from attending day care due to restricts set in place during pandemic. A recent study reported that children without signs or symptoms of COVID-19 rarely tested positive for SARS-CoV-2 RNA, even in a region with a very high burden of COVID-19.[28] Excluding children with symptoms of respiratory tract infections from attending may be important in reducing the risk of undetected chains of transmission of SARS-CoV-2 in the day care centre setting. This may prove to be a challenge in the coming winter, when upper airway infections in children are expected to occur frequently.

A strength of this study was the distribution of participating day care centres throughout Hesse (figure 1), including facilities from both metropolitan and rural regions, with diverse socioeconomic and migration backgrounds, as well as varying activity of SARS-CoV-2. Individual participation in the study by families as well as day care staff was voluntary. This likely caused selection bias and also may have caused the “Hawthorne effect”, as families who decided to participate in the study may also exhibit a more defensive behaviour in the pandemic with weekly reinforcement. Information on the study and instructions were only provided in German, with an instructional video available with English subtitles, but no other languages were made available. This likely led to an underrepresentation of families with native languages other than German. It is also possible, that the low incidence of SARS-CoV-2 infection reflected the effectiveness of general infection control measures still in effect at the time (mask requirements etc.). Furthermore, in a higher incidence context, a role of young children as transmission vehicles might be more apparent.

In conclusion, the detection of either respiratory or gastrointestinal shedding of SARS-CoV-2 occurring in day centres with a local incidence up to 66 cases / 100,000 inhabitants was rare during the pandemic, with prevention measures in place. Further studies should examine whether this is also the case for a setting with higher activity of SARS-CoV-2 infections.

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Statistical Consultation

The Hessian State Office for Statistics provided a representative sample of day care centres in Hesse.

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Potential conflicts of interest

S.C. has received speaker´s fees from Roche Diagnostics.

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Figure Legends

Figure 1: Distribution of participating day care centres throughout in the state of Hesse, Germany. The accumulation of centres in the south of Hesse corresponds to a metropolitan region that has the highest population density of the state.

Figure 2: Number of samples provided by study participants, by week. Summer recess occurred from calendar week 28 to 33. Recruitment of centres was not complete in week 25.

Figure 3: Results of testing for GAPDH mRNA in buccal mucosa swabs (percentage of positive samples and 95% CI are shown)

Table 1: Numbers of samples that were tested in the study, by age group

Age of study participants	Number of study participants	Number of samples		
		Buccal mucosa swab	Anal swabs	Total
0 to 2 years	120	691	634	1,325
3 to 5 years	661	3,820	3,142	6,962
6 to 8 years	44	257	214	471
Age unknown (children)	34	173	126	299
19 to 64 years	376	2,425	1,791	4,216
Total	**1,235**	**7,366**	**5,907**	**13,273**

Table 2: Results from the questionnaires

	Children: Caregiver indicating yes (out of 557 participants who returned the questionnaire)	Day care staff: Number of participants indicating „yes“ (out of 256 staff members who returned the questionnaire
Was your child / Were you diagnosed with a SARS-CoV-2 infection (COVID-19) during the study period outside of the study?	*0 (0%)*	*0 (0%)*
Was your child / Were you exposed to SARS-CoV-2 (COVID-19) in the household during the study period?	*0 (0%)*	*0 (0%)*
Did your child / Did you have contact with a person with SARS-CoV-2 (COVID-19) during the study period?	*8 (1.4%)*	*7 (2.7%)*
Did your child / did you spend time abroad during the study period?	*118 (20.8%)*	*45 (17.6%)*
Did your child / did you stay in a country that was designated a “risk area” of COVID-19 by the Robert Koch institute during the study period?	*5 (0.9%)*	*7 (2.7%)*

Figure 1

Figure 2

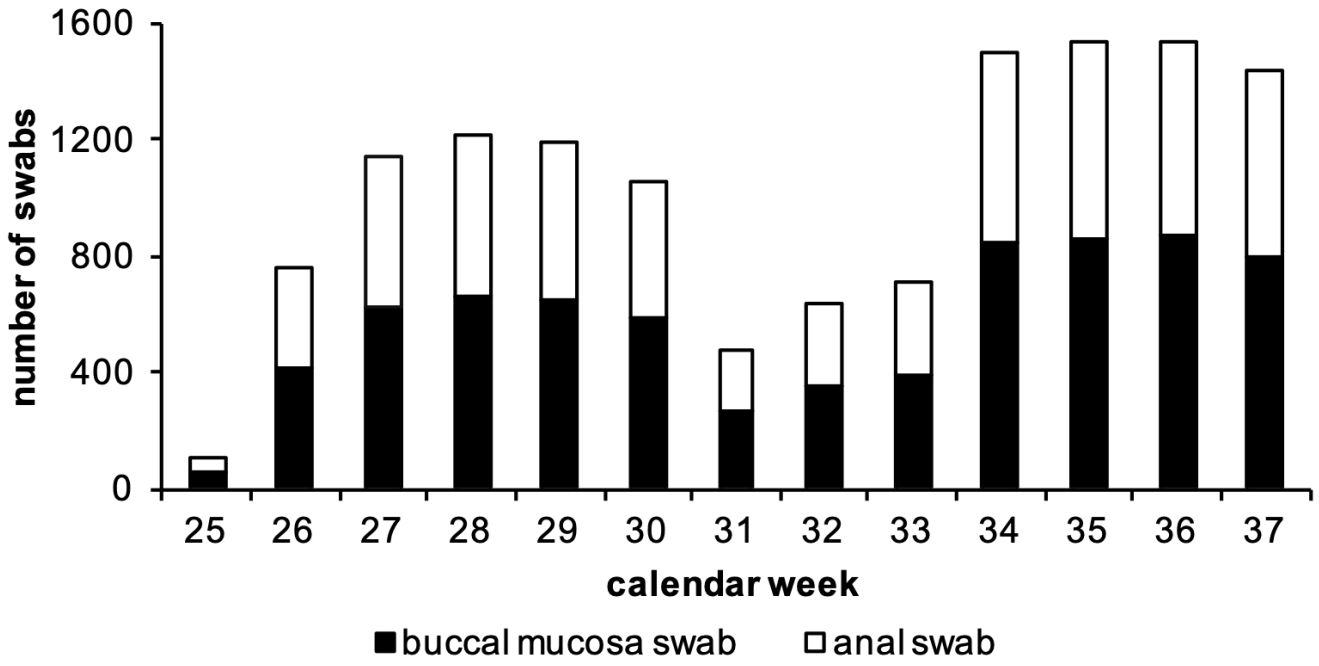
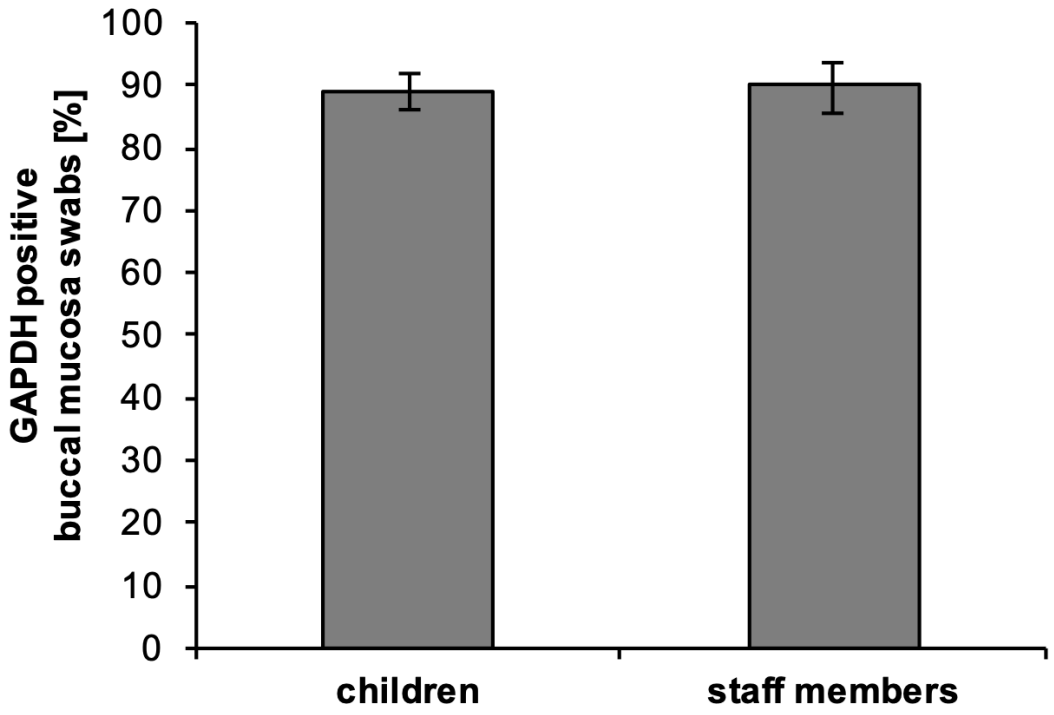

1600
1200
800
400
0
number of swabs
25
26
27
28
29
30
31
32
33
34
35
36
37
calendar week
buccal mucosa swab
anal swab

Figure 3


GAPDH positive buccal mucosa swabs [%]
100
90
80
70
60
50
40
30
20
10
0
children
staff members