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Comparing susceptibility and contagiousness in concurrent outbreaks with a non-VOC and the VOC SARS-CoV-2 variant B.1.1.7 in daycare centers in Hamburg, Germany

Jacqueline Nakel a,1, Alexis Robitaille a,1, Thomas Günther a,1, Lorenz Rosenau c, Manja Czech-Sioli b, Anita Plenge-Bönig d, Silja Bühler d, Andreas Wille d, Elke Jakubowski e, Susanne Pruski c, Manuela Wahlen c, Daniela Indenbirken a, Dominik Nötz b, Marc Lütgethetmann b, Martin Aepfelbacher b, Adam Grundhoff a,***, Benjamin Grolle e, **, Nicole Fischer b.

a Leibniz Institute for Experimental Virology (HPI), Hamburg, Germany
b Institute for Medical Microbiology, Virology and Hygiene, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
c Bezirksamt Altona, Fachamt Gesundheit, Freie und Hansestadt Hamburg, Germany
d Division of Hygiene and Infectious Diseases, Institute for Hygiene and Environment, Hamburg, Germany
e Department of Infectious Diseases and Public Health Services, Authority of Social Affairs, Hamburg, Germany

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ABSTRACT

We describe two outbreaks of SARS-CoV-2 in daycare centers in the metropolitan area of Hamburg, Germany. The outbreaks occurred in rapid chronological succession, in neighborhoods with a very similar sociodemographic structure, thus allowing for cross-comparison of these events.

We combined classical and molecular epidemiologic investigation methods to study infection entry, spread within the facilities, and subsequent transmission of infections to households. Epidemiologic and molecular evidence suggests a superspreading event with a non-variant of concern (non-VOC) SARS-CoV-2 strain at the root of the first outbreak. The second outbreak involved two childcare facilities experiencing infection activity with the variant of concern (VOC) B.1.1.7 (Alpha). We show that the index cases in all outbreaks had been childcare workers, and that children contributed substantially to secondary transmission of SARS-CoV-2 infection from childcare facilities to households. The frequency of secondary transmissions in households originating from B.1.1.7-infected children was increased compared to children with non-VOC infections. Self-reported symptoms, particularly cough and rhinitis, occurred more frequently in B.1.1.7-infected children.

Especially in light of the rapidly spreading VOC B.1.617.2 (Delta), our data underline the notion that rigorous SARS-CoV-2 testing in combination with screening of contacts regardless of symptoms is an important measure to prevent SARS-CoV-2 infection of unvaccinated individuals in daycare centers and associated households.

1. Introduction

During the first wave of the SARS-CoV-2 pandemic in Germany, ranging from March until May 2020, childcare facilities were closed in most federal states. In contrast, most of the facilities remained open during the second and beginning of the third waves, mid-October 2020 to February 2021 and February to May 2021, respectively, although infection rates among children and adolescents had substantially increased. Operation at reduced capacity, separation of children into smaller cohorts, and implementation of hygiene measures were...
undertaken to contain infections and keep facilities open. However, unlike in school settings, neither employees nor children were mandated to wear a mask in daycare centers. Nevertheless, the overall rate of daycare centers in Germany that had to be closed due to recognized infections increased from 1.7% by the end of January 2021 to a peak of 5.8% by the end of April 2021 (Autorengruppe, 2021). Considering the high incidence rates among children and adolescents in the second and third waves of the pandemic, it was controversially discussed how these age groups contribute to the SARS-CoV-2 infection dynamics.

To date, only a few case reports have been available, focusing on systematic studies on SARS-CoV-2 infection events in daycare centers (Autorengruppe, 2021). Moreover, some geographically limited studies were conducted (Ehrhardt et al., 2020; Haag et al., 2021; Hoehl et al., 2021; Hoehl et al., 2021; Lübke et al., 2021; Thielecke et al., 2021). However, most of these surveillance studies were performed in periods with low overall incidence rates in Germany. Only a single study, describing three independent outbreaks with the VOC B.1.1.7, addresses the effect of newly emerging variants (Joenenbach et al., 2021). This study indicates that susceptibility as well as transmissibility of children and adults might converge with the emergence of new, more infectious variants. Given the variant B.1.617.2 and no available vaccination in children under 12 years of age, it is of significant interest to investigate transmissions in children and by children in households and reconsider hygiene measures in daycare centers. We report on three transmission clusters in daycare centers in Julin et al., 2021, occurring in the Hamburg metropolitan region. We compare transmission events, the number of infected individuals, and the development of symptoms in individuals experiencing a non-VOC SARS-CoV-2 outbreak and a VOC B.1.1.7 outbreak.

2. Methods

2.1. Sample collection

In light of the positive SARS-CoV-2 PCR test results of the index cases, nasopharyngeal samples were collected from every child and childcare worker in the respective daycare facility to find potentially associated infections. Serial testing procedures were performed to collect samples. Single samples were collected in medical practices. Index cases and all contact persons were contacted regularly by public health workers to ask for monitoring of symptoms. Household contacts were offered voluntary PCR tests. The qRT-PCRs of the SARS-CoV-2 specimens were performed in accredited laboratories in Hamburg, Germany.

2.2. qRT-PCR based SARS-CoV-2-testing

Samples were sent to the University Medical Center Hamburg-Eppendorf for independent SARS-CoV-2 qRT-PCR confirmation and PCR-genotyping (Corman et al., 2020; Puelles et al., 2020). PCR-genotyping for the SARS-CoV-2 variant of concern (VOC) B.1.1.7 was performed by using specific primer/probe (Brehm et al., 2021) sets for B.1.1.7 as published (Norz et al., 2021).

2.3. SARS-CoV-2 amplicon sequencing and bioinformatics analyses

Amplicon sequencing and bioinformatics analysis were performed as recently published (Brehm et al., 2021; Gunther et al., 2020). Library generation was performed using the CleanPlex SARS-CoV-2 Panel (Paragon Genomics, CA, USA). Merged reads were filtered for a minimum base quality of 20 and aligned to NC_045512.2 using minimap2 (Li, 2018) with default settings for short read alignment. Major variants (≥50% allelic frequency) were called using FreeBayes Bayesian haplotype caller v1.3.1 (Garrison and G, 2012) with ploidy and haplotype independent detection parameters to generate frequency-based calls for all variants supported by a minimum coverage of 10 (–K 0.5-min-coverage 10). Resulting variants were annotated using ANNOVAR (Wang et al., 2010) and consensus sequences were generated using in-house scripts. Pangolin lineage and Nextstrain clade assignment of consensus sequences were performed using the pangolin (https://github.com/cov-lineages/pangolin) and Nextclade (https://github.com/nextstrain/nextclade) packages. Phylogenetic analysis and tree visualization were performed using Nextstrain which relies on maximum likelihood ancestral state reconstruction of discrete traits in combination with inferred probability distributions of ancestral state of each node (Hadfield et al., 2018). The sequences have been uploaded to GISAID (see Table S4 for accession numbers).

3. Results

The two outbreaks in daycare centers occurred in the same middle-class neighborhood involving three independent daycare centers, at a time when overall incidence rates were high and B.1.1.7 prevalence was increasing in the city of Hamburg (Fig. 1A). At the time of the outbreaks (end of January), the second wave was about to decline, and the 7-day incidence rates ranged between 70 and 100 cases per 100,000 individuals. Although children and adolescents were highly affected during the second and the third wave of the pandemic in Germany, 7-day incidence rates among children aged 0–5 years in Hamburg were still below the overall average (40–50 cases per 100,000 individuals in Germany). Restrictions in different areas of public life as well as for private meetings started by mid of December 2020 (Fig. 1B). Schools and daycare facilities worked under emergency conditions with reduced capacities.

3.1. Infection series in the three childcare facilities

The first case related to childcare facility 1 was a childcare worker who reported to health authorities with a positive PCR test result (C1, 17.7). The individual had been symptomatic and therefore had taken sick leave two days before the positive test result. Prior the occurrence of symptoms, the individual had been responsible for a group of children below the age of 3 in the nursery department, and additionally had supervised the dormitory for the entire daycare center’s nursery. Consequently, a strict quarantine of 14 days was ordered for all children attending the nursery and close contacts among the childcare workers. At the time of the outbreak there was no possibility to shorten the quarantine through PCR testing. In the following days, two of the quarantined staff developed symptoms and tested positive. Rapid antigen tests were also performed on four additional childcare workers with three of them testing positive with results being confirmed by qRT-PCR. As these childcare workers took care of groups from the elementary cohort (children aged 3–6 years), a second quarantine was ordered. Within 8 days after the index case turned out to be SARS-CoV-2 positive, 8 out of 14 childcare workers and 22 out of 34 children tested positive (Fig. 2A). Due to the high infection rates (Facility Secondary Attack Rate (SAR) = 63.3%) the outbreak was considered a potential superspreading event. Because of the high number of positive PCR test results compared with previous outbreaks in childcare facilities, all contact persons of the second generation affected by the outbreak were offered voluntary PCR testing. This revealed several household transmissions originating from childcare workers as well as children. While childcare workers accounted for 5 infections of the second generation (2 parents and 3 children), children attending the daycare center infected a total of 15 household contacts (14 parents and one sibling). Shortly after the outbreak in F1, another SARS-CoV-2 outbreak was reported in a childcare center in the same city district. The index case of facility 2 (F2), a childcare worker taking care of children in the nursery, was tested positive by PCR three days after symptom onset while being on sick leave. PCR genotyping indicated the presence of characteristic mutations for the VOC B.1.1.7 (S:69-70:H-V; S:501:N:Y) and the first outbreak of VOC B.1.1.7 in an educational institution in Hamburg. As a
consequence the whole daycare facility was quarantined. qRT-PCR revealed that due to strict separation between the nursery and elementary cohorts only contacts from the nursery cohort showed SARS-CoV-2 infections, while the elementary school remained unaffected. In total, 6 out of 14 childcare workers and 10 out of 71 children tested positive for SARS-CoV-2 by PCR (Facility SAR = 19.8%, Fig. 2B). The outbreak included infections in the households of children (11 parents, 2 siblings) and childcare workers, which occurred within 23 days after the index case turned out to be SARS-CoV-2 positive (one parent, one partner, and one child).

Due to infections in the household, the partner of one of F2's childcare workers, who is employed at F3, was infected and served as the index case at F3 only 2 days after the index case of facility 2. Consequently, a two-week quarantine was ordered for all employees and children during which 8 out of 42 children and 5 out of 17 employees tested positive (facility SAR = 23.3%, Fig. 2B). These 13 persons were responsible for 15 positively tested household contacts. Transmissions from children included 7 parents and 3 siblings while those from employees included 2 parents, 2 partners, and one child.

3.2. Viral genotypes in the three childcare facilities

Based on the assumption that the outbreak at F1 might have been a superspreading event and that outbreaks at F2 and F3 involved the VOC B.1.1.7, whole-genome sequencing (WGS) was performed. For F1 a total of 23 samples were available of which 20 yielded high-coverage genotypes (the index case and 19 contacts of the first generation (4 childcare workers and 15 children, Fig. 3A, F1-1 – F1-22)). Compared to the SARS-CoV-2 reference (NC_045512.2), all samples exhibit a set of 17 dominant mutations defining the index sequence (F1_1-A index). In three samples, F1_14-C, F1_20-A and F1_22-C, single nucleotide polymorphisms (SNPs) were detected with F1_14-C and F1_20-A showing 2 SNPs each with 70% and 100% variant frequency, respectively. F1_22-C contains one SNP with 10% variant frequency (Fig. 3A). The pangolin lineage of the 20 samples was determined as B.1.1. A total of 14 samples from childcare F2 and F3 were analyzed by WGS: 6 samples from childcare workers (4 and 2 for F2 and F3, respectively) and 8 samples from children (6 and 2 for F2 and F3, respectively). Index samples were not available for sequencing. Data analysis confirmed the presence of the VOC B.1.1.7 and identified an identical set of mutations shared by all samples (Fig. 3A), thereby confirming that the two outbreaks are directly linked.

To investigate whether similar genotypes could be identified beyond

![Fig. 1. A) SARS-CoV-2 7-day incidences (per 100'000 individuals), percentage of B.1.1.7 and hygiene measures (B) in the city of Hamburg, Germany, October 19, 2020–February 18, 2021. SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; W: calendar week. (Data source: Hamburg Institute for Health and Environment).](image-url)
the daycare centers, data were compared with sequences deposited in GISAID (gisaid.org), as well as with the dataset of the Hamburg-Surveillance program, an endeavor which systematically monitors SARS-CoV-2 variants in the Hamburg metropolitan area (Surveillance). The generated phylogenetic tree recapitulates the association of samples from F1 with B.1.1 and F2 and F3 with the variant B.1.1.7 (Fig. 3B). A zoom-in view reveals that three samples share the exact genotype of the outbreak at F1 (Fig. 3B). Interestingly, one of these samples was collected 10 days after the positive test of the index case and originated from a location very close (<1 km) to the daycare center. We performed the same analysis in the context of F2 and F3. Similarly, we identified three samples with the identical genotype as seen in the samples from F2 and F3 (Fig. 3C). In addition, we identified four samples that had 1-4 additional single nucleotide polymorphisms (SNPs) and two samples lacking one of the observed nucleotide exchanges. All samples with the prototypical outbreak genotype were collected in the city of Hamburg. However, we were unable to trace any of the samples to the outbreaks. The fact that most of the closely related samples (with the exception of three samples with identical genotype to F1 sequences) show additional mutations and were found relatively far from the facilities suggests that all outbreaks were contained at the level of second generation contacts.

3.3. Comparison of symptoms and numbers of secondary contact persons

As SARS-CoV-2 variants differ between F1 (B.1.1, non-VOC) and F2/3 (B.1.1.7, VOC), we compared self-reported symptoms among the index cases and first-generation contacts. We differentiated between children and childcare workers as well as between non-VOC and VOC facilities. In F1, fever, cough, and cold were the most frequently reported symptoms in both, children and childcare workers with 52% reported fever, 46% cough and 49% cold. While loss of taste/smell was also reported frequently among the childcare workers (77.8%), young children were rarely affected (4.5%). In general, children reported less symptoms with 5 children being symptom-free (Table 1, Table S1). Interestingly, among the individuals infected with B.1.1.7 in F2/3, the rate of reported symptoms was comparable among childcare workers and children (Table 1, S2 and S3). Fever was more...
Fig. 3. Heatmap and phylogenetic tree representing the viral genotypes detected by SARS-CoV-2 whole-genome sequencing of samples from the three childcare facility outbreaks in Haag et al., 2021 – February 2021. (A) The heatmap shows the position (left to right), the identity (lower row), and the frequency (color code) of substituted nucleotides identified by SARS-CoV-2 genome amplicon sequencing. The annotation to the pangolin lineage is depicted in the left column. Variants are relative to the reference strain initially found in Wuhan (NC_045512.2). White rectangles mark positions which were not covered by sequencing. Low coverage regions (<5 reads) are marked by blue dots. The open reading frame (ORF) as well as the type of amino acid change of the respective nucleotide position is highlighted as color code in the top rows of the heatmap. (B + C) Time-based phylogenetic tree highlighting the samples of the outbreaks in childcare facility 1 (B) and in childcare facilities 2 and 3 (C) in the context of German SARS-CoV-2 isolates. The phylogenetic tree was generated from the data of the childcare facility outbreaks, the Hamburg-Surveillance Database, and data available through GISAID (gisaid.org). The samples are color-coded by the allocation to children (blue) and childcare worker (yellow). The geographical distance of the related samples to the childcare facility is indicated as below 1, below 10 and below or above 20 km. AA: amino acid; A: adult; C: child; F: facility; km: kilometer; ORF: open reading frame; SNV: single nucleotide variant.
common in children (50%) than in childcare workers (16.7%), although overall rates were lower compared to F1 (35.3% vs. 52%). In F2 and F3, only two children and one childcare worker remained symptom-free.

We also analyzed SARS-CoV-2 household transmissions of the non-VOC and the VOC outbreaks. As the total number of second-generation contacts was unknown, SAR calculation was not possible. To compare non-VOC and VOC scenarios and detect potential differences between children and adults, we calculated the ratio of infected contact persons per condition (Table 2). While the ratios of infected second-generation contacts were comparable between non-VOC children (0.68) and non-VOC childcare workers (0.63), substantial, however not statistically significant, differences in the context of the VOC outbreaks were observed. The ratio of contacts infected by the VOC childcare worker was with 0.75 only slightly increased compared to the ratios of the non-VOC outbreak. In contrast, one VOC-affected child infected on average 1.3 household contacts indicating that with the occurrence of more infectious variants children contribute to SARS-CoV-2 transmissions more frequently.

4. Discussion

The study presented here provides the first comparative investigation of non-VOC- and VOC-induced SARS-CoV-2 outbreaks in childcare facilities. The fact, that the facilities were located within the same district of Hamburg, ministered to children with similar social-demographic background, and the outbreaks occurred within a short timeframe allow this comparison. Epidemiologic and molecular evidence including a SAR of 63.3%, suggests that the outbreak at F1 resulted from a superspreading event with the SARS-CoV-2 lineage

Table 1
Summary of self-reported symptoms of persons affected by the three childcare outbreaks (index and K1 contacts) in the city of Haag et al., 2021–February 2021. In the table, non-VOC/VOC infected children and non-VOC/VOC infected childcare workers are distinguished.

| strain     | group             | n | %  | n | %  | n | %  | n | %  | n | %  |
|------------|-------------------|---|-----|---|-----|---|-----|---|-----|---|-----|
|            |                   |   | fever |   | cough |   | cold |   | loss of taste/smell |   | shortness of breath |   | kidney pain |   | stay on ICU |   | fatigue |   |
| B.1.1 (non-VOC) | children          | 11 | 50.0 | 8 | 66.7 | 8 | 55.6 | 6 | 53.8 | 3 | 100 |
| B.1.1.7 (VOC) | childcare worker | 6 | 66.7 | 7 | 77.8 | 7 | 77.8 | 1 | 77.8 | 11 | 77.8 |
| B.1.1 (non-VOC) | children          | 7 | 38.9 | 10 | 55.6 | 10 | 55.6 | 1 | 53.8 | 6 | 38.9 |
| B.1.1.7 (VOC) | childcare worker | 4 | 30.8 | 6 | 53.8 | 7 | 53.8 | 6 | 46.2 | 1 | 7.7 |

B.1.1.7. In contrast, the outbreaks in F2/F3 represent a series of infection events with the SARS-CoV-2 VOC B.1.1.7. The SAR values (19.8%, F2 and 23.3%, F3) are lower, but are consistent with previous reports of B.1.1.7-associated outbreaks where SAR calculation ranged from 17 to 37% (Loenenbach et al., 2021).

Interestingly, childcare workers were identified as index cases in all three facilities. This is consistent with previous national and international reports of childcare center outbreaks, which all had originated from childcare workers (Hoehl et al., 2021; Lopez et al., 2020; Okarska-Napierala et al., 2021). However, these studies described outbreaks in the early phase of the pandemic, prior to the emergence of VOC SARS-CoV-2 strains. As children often remained asymptomatic during non-VOC SARS-CoV-2 infection, they might have been only sporadically identified as index cases (Davies et al., 2020; Laws et al., 2021). With the emergence of new, more infectious SARS-CoV-2 variants, such as B.1.1.7 or B.1.617.2, children may develop symptoms more frequently, potentially facilitating their identification as index cases. This observation is consistent with reports from the U.K. Office for National Statistics, which reported a higher proportion of persons who tested positive for the B.1.1.7 variant with symptoms compared with infected persons without the variant. Specifically, loss of taste and smell were reported less frequently and cough, sore throat, and myalgia were reported more frequently. However, two studies that examined the difference between B.1.1.7 infections and non-B.1.1.7 infections reported no significant difference in symptoms in adults or children (Graham et al., 2021; Meyer et al., 2021).

Although many childcare workers and children were affected by the SARS-CoV-2 outbreak at F1, infection of household contacts occurred only sporadically. On average, one child or childcare worker at the non-VOC facility was responsible for 0.68 and 0.63 household infections, respectively. While the average number of household infections originating from a VOC-infected childcare worker was 0.75, only slightly higher than that of non-VOC childcare workers, a VOC-infected child infected on average 1.3 household contacts. The observation of more frequent transmission in households among persons infected with B.1.1.7 infections and non-B.1.1.7 infections reported no significant difference in symptoms in adults or children (Graham et al., 2021; Meyer et al., 2021).

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Table 2
Summary of secondary contact persons affected by the three childcare outbreaks in the city of Haag et al., 2021–February 2021. In the table, non-VOC/VOC infected children and non-VOC/VOC childcare workers are distinguished. The ratio of infected secondary contact person per non-VOC/VOC child/childcare worker is calculated by dividing the infected secondary contacts by the total number of infected persons. *Index case is not included.

| strain | group             | facility | total number of infected persons | infected K2 contacts | ratio |
|--------|-------------------|----------|----------------------------------|----------------------|-------|
| B.1.1  | children          | facility | 22                               | 15                   | 0.68  |
| (non-VOC) | childcare worker | 1        | 8                                | 5                    | 0.63  |
| B.1.1.7| children          | facility | 10                               | 13                   | 1.3   |
| (VOC)  | childcare worker | 1        | 8                                | 10                   | 1.25  |
|        |                   | sum      | 18                               | 23                   | 1.28  |
|        |                   | facility | 6                                | 3                    | 0.5   |
|        |                   | 2        |                                   |                      |       |
|        |                   | facility | 5                                | 5                    | 1.0   |
|        |                   | sum      | 11                               | 8                    | 0.75  |
outbreaks and associated household infections (Madewell et al., 2020). Further limitation of this study are the self-reported symptoms of children and childcare workers and the lack of WGS from index cases in F2 and F3 and second-generation contacts. However, the epidemiologic investigation and the uniform genotypes of the available samples demonstrate a link between infection events. In addition, most second-generation infections could be linked to the outbreaks in the facilities based on their temporal classification.

Overall, our study provides new insights into the role of outbreaks in daycare centers during the SARS-CoV-2 pandemics. To our knowledge, it is the first study that, due to the timing of outbreaks and the geographical proximity of daycare centers, allows to compare VOC and non-VOC outbreaks. Altogether, revisiting hygiene measures should be the first study that, due to the timing of outbreaks and the associated household infections (Madewell et al., 2020).

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