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Analysis of COVID-19 Infection Chains in a School Setting: Data From a School-Based rRT-PCR–Gargle Pool Test System

Magdalena Gruendl MD, MPH[1,2], Parastoo Kheiroddin MSc[2], Michael Althammer BSc, MSc[2], Patricia Schöberl[2,3], René Rohrmanstorfer MSc[4], Daniel Wallerstorfer PhD[4], Andreas Ambrosch MD[5] and Michael Kabesch MD[2,3]

[1]Public Health Office, Cham, Germany; [2]Department of Pediatric Pneumology and Allergy, University Children's Hospital Regensburg (KUNO) at the Hospital St. Hedwig of the Order of St. John and the University of Regensburg, Regensburg, Germany; [3]Member of the Science and Innovation Campus Regensburg (WECARE) at the Hospital St. Hedwig of the Order of St. John, Regensburg, Germany; [4]NOVOGENIA Limited, Eugendorf, Austria and [5]Institute of Laboratory Medicine, Microbiology and Hygiene, Hospital of the Order of St. John, Regensburg, Germany

Brief Report

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Corresponding author:
Michael Kabesch,
Email: michael.kabesch@barmherzige-regensburg.de.

Abstract

Background: School testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was implemented in some countries to monitor and prevent SARS-CoV-2 transmissions. Here, we analyze infection chains in primary schools and household members of infected students based on systematic real-time reverse-transcriptase polymerase-chain-reaction (rRT-PCR)–gargle pool testing.

Methods: Students and school staff ($N = 4300$) of all 38 primary schools in the rural county of Cham, Germany, were tested twice per week with a gargle pool rRT-PCR system from April to July of 2021. Infection chains of all 8 positive cases identified by school testing were followed up.

Results: In total, 8 positive cases were found by gargle pool PCR testing based on 96,764 school tests. While no transmissions occurred in the school setting, 20 of 27 household members of the 8 cases tested positive. The overall attack rate was 74.1% in families.

Conclusions: No school outbreaks occurred during the study period. All cases but 1 were initially picked up by school testing. No transmission from school to families was observed.

The coronavirus disease 2019 (COVID-19) pandemic led to a considerable disruption of regular school operations in many countries, which impaired the development and quality of life of many children. To maintain safe school operations, corona testing has become a helpful tool in addition to existing hygiene concepts.[1] With real-time reverse-transcriptase polymerase-chain-reaction (rRT-PCR) tests, the gold standard in diagnosing SARS-CoV-2, infections can be detected quickly and with much higher sensitivity than antigen tests.[2]

We and others have shown that regular and systemic pool rRT-PCR testing, using gargle fluid is a sensitive, efficient, and inexpensive alternative to antigen-test for monitoring of SARS-CoV-2 infections and at the same time, significantly reduces the risk of transmission if high participation rate is achieved.[3–5] Here, we evaluate if such a sensitive rRT-PCR based test concept can pick up infections when brought into schools and disrupt infection chains even before infections can spread in the school setting. Thus, we concomitantly analyzed infections in the school and private setting based on a large number of school-based tests in a Bavarian County in which school children aged 6-10 and school staff participated in large numbers in the WICOVIR (**W**here **I**s the **CO**rona **VIR**us) project between April and July of 2021.

Methods

Study Setup

The study took place from April 12, 2021, until July 31, 2021, in Cham County, a large rural county with 128,094 inhabitants as previously described.[1] All 38 primary schools in the county participated, with approximately 4300 students (ages 6-10 y) and teachers and staff testing twice per week in 215 pools (Figure 1). Teachers and staff made up approximately 5% of all tested individuals. During the study, mitigation strategies for all Bavarian schools included the mandatory use of FFP2 masks for teachers and surgical masks for students, keeping distance, washing hands frequently, and increasing fresh air circulation in classrooms.

The WICOVIR project was approved by the ethics committee of the University of Regensburg (file number 21-2240_2-101). Participants and parents of minors were asked for

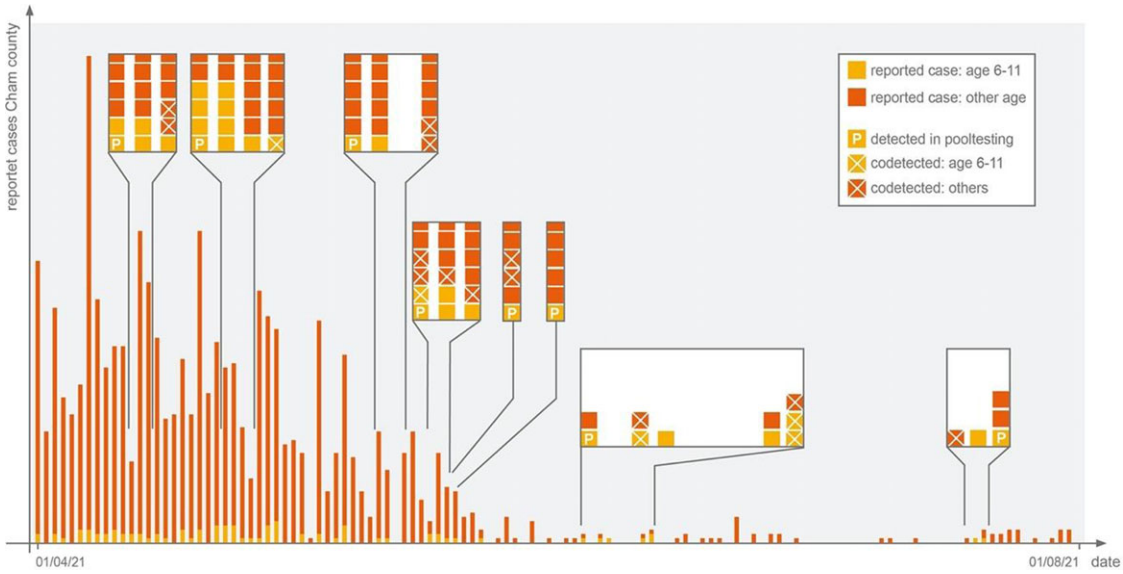

Figure 1. Timeline of the positive pools in Cham County. Development of reported cases in Cham County over the study period and pool-PCR tested positive students and their positive household members.

consent. All study procedures and protocols are available online (www.we-care.de/wicovir), have been published,[1] and are summarized in online Supplementary Materials 1.

Wicovir Gargle Pool rRT-PCR Testing in Schools

In brief, all children with written parental consent gargled with tap water at home before coming to school and pooled their samples in schools. Sample pools, which mostly consisted of 1 class and their respective teacher (and staff), were transported to the laboratories, where they were analyzed within the same day with rRT-PCR. First, NOVOGENIA GmbH (Eugendorf, Austria) tested the pools; from June 4, 2021, pools were tested in the Regensburg laboratory as previously described.[1] In the case of a positive result, pools were always depooled in the Regensburg laboratory, usually within the same day, and results were immediately transmitted to the public health office in Cham.[1] For the few children and teachers who did not participate in the study, an alternative antigen test was mandatory to attend school.

Contact Tracing

The family of an index person was contacted no later than the following morning after the positive test result by the Cham public health office. Data on viral load (Ct-values), symptoms, duration of symptoms, risk factors, and any hospitalization within the family were collected in a Web-based software (XIMA® FORMCYCLE, Dresden, Germany).

Detailed information on all index and contact persons could only be collected on residents of Cham County, because the public health office is only authorized to contact and trace permanent residents of that county. For residents outside the county, only basic information was available. Records of the public health office were analyzed for further positive test results of all inhabitants of Cham County in the age range of school testing participants.

Results

Pool rRT-PCR testing was performed from April 12, 2021, until the end of the school year on July 31, 2021, in 38 schools with a total of 4300 students, teachers, and staff, of which a maximum of 3860 participated in any single test round. In total, 96,764 test samples were pooled and analyzed in the study period in the county on 47 test days, cumulating in 6115 pool rRT-PCRs and 94 single PCRs for depooling. In total, 9 positive pools contained 11 individual positive samples. Of these, 2 individual test results were ambiguous and individual retesting revealed that children were negative (technical error). A further 2 positive samples resulted from children who previously had COVID-19 and were still shedding virus particles and participated in pool testing despite being instructed to refrain from testing for 3 mo. The remaining 7 individual SARS-CoV-2 positive cases were all students and did not cluster in a specific school but were from 6 different schools throughout the county.

In another case, a student was identified as a contact person due to a positive family member 1 d after participating in the (negative) pool testing and tested positive at the local test center (Ct- value 25,2/26,6). The student's backup sample from the negative pool testing was still available and individual rRT-PCR of that sample revealed Ct-values of 36 and 38 (for both measured genes), which is just beyond the detection limit of the WICOVIR rRT-PCR gargle pool system. This student was also included in the transmission analysis.

To exclude that other infections were missed by school pool testing, records of the public health office were analyzed for further (mandatory) reports of positive test results of inhabitants of Cham County in the age range of participants of the school testing. Some additional children were tested positive at the local test center or the pediatricians' or physicians' office during the study period, but all these children were contact persons to a known case and were in quarantine; thus, they did not attend school during their infectious period and could not be tested in pool testing. They were not relevant for this study and, therefore, not included.

Overall, there were 35 persons involved in the 8 cases we report: 8 students and 27 household members of whom 20 were tested positive subsequently (Figure 2). In all these cases (except case 8), the student who tested positive in the school was the first case detected in their household. For the 24 household members who lived in Cham County, detailed data could be collected for this

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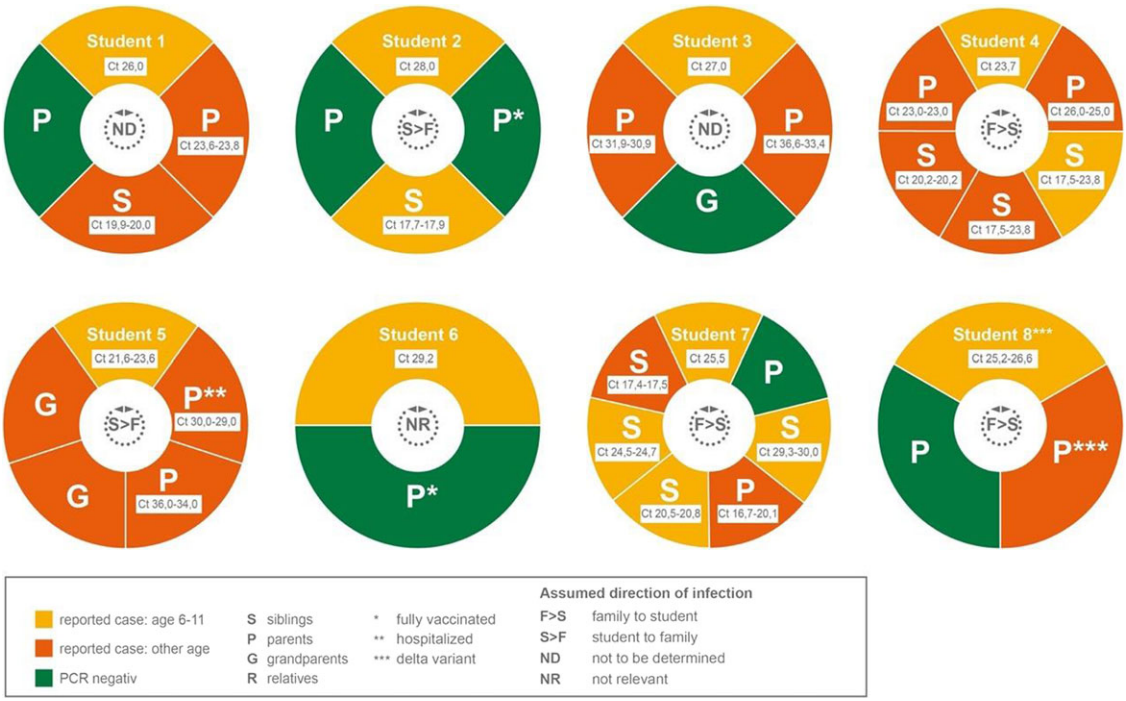

Figure 2. Direction of transmission within the household. The infection rings include all persons (n = 35) within the household including the ones (n = 3) who do not reside in Cham County.

study (Supplementary Materials 2). The 3 people who resided outside Cham district were grandparents, whom the families visited for a minimum of 2 d before the infection; therefore, they were handled like household members (student 3 and student 5). This constitutes an attack rate of 74.1%. See also Supplementary Materials 2 for details. In contrast to the high attack rate within the family, no additional positive cases were detected in the schools (secondary attack rate of 0%). Therefore, we could exclude the schools as the likely source of the infections.

Direction of transmission within the household was independently assessed by 3 reviewers (medical officer, pediatrician, hygiene expert) based on the timing of positive tests and CT values as well as occurrence of symptoms (Supplementary 2; Figure 2). There was strong agreement in 2 cases that the student was the primary case and transmitted SARS-CoV-2 to the family (cases 2 and 5). In cases 4, 7, and 8, there was strong agreement that students were infected by other family members. In cases 1 and 3, transmission of infection could not be determined, and it remains unclear who was the first infected person within the household. In case 6, the student did most likely not infect his/her fully vaccinated parent, but this could not be proven as testing of vaccinated contact persons was not mandatory at the time and, thus, could not be performed.

Discussion

The WICOVIR school testing identified all SARS-CoV-2 positive children in the county in a timely manner when infection rates in the county were around 200 cases per wk per 100,000 inhabitants. During the surveillance time, there were no transmissions within the schools observed.

The setting of this study was unique in that all primary schools in an individual county participated at the same time, and all further data records for SARS-CoV-2 infections in the county were available. The analysis revealed no infections were missed by the gargle pool rRT-PCR system, except 1 case as described above. No false positive tests occurred. How much more inferior antigen tests perform in the early detection of cases has previously been reported.[5]

We and others argue, that a highly sensitive test system is the prerequisite to avoid outbreaks in schools as the timing for isolation is crucial.[5–7] Here, the isolation process was optimized through close collaboration between the test center and the public health office, from the day after the pooling at the beginning of the project to the same afternoon as the pool testing after week 10 of the project. Due to the fast isolation of cases with high CT values and a low viral load, further quarantine of contact persons in the schools could be avoided and still no follow-up infections were observed.

With our comprehensive data base, we investigated the possible route and direction of SARS-CoV-2 transmission (Figure 2; Supplementary Materials 2). While schools could be excluded as a source of transfection, it was not always clear who brought the infection into the family. In 3 cases, it seems most likely that a student may have been infected outside the school and outside the family, transmitting the infection to the family. However, it also could not be excluded that other (adult) family members were the source of the infection in the family, as shown in previous studies from the first SARS-CoV-2 wave in spring of 2020.[8]

To protect schools from uncontrolled SARS-CoV-2 transmissions, pool PCR screening was recommended to be implemented at a low initial incidence, at much lower incidence rates as reported here, as the effects of pool PCR screening were suspected to lose power as incidence increases.[9] However, we found that the protective effect of the screening does not only depend on incidence but also on the logistics of the testing setup. New, more contagious variants lead to decreased incubation times, higher infection rates, and higher incidence, which can be countered by more frequent testing

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combined with faster communication of results and implementation of isolation.[10]

One could argue that conclusions about transmission are only based on 8 cases in a low incidence window of the pandemic. Indeed, this is a limitation in the analysis and interpretation. On the other hand, transmission can only be studied properly in times of low incidence as cryptic sources of infection can widely be excluded. However, the drawback for this is that many screenings have to be performed to identify 1 positive case early (eg, >15,000 tests in our setting). Early identification is needed to follow transmission prospectively, as it was possible in 7 of 8 cases. Also, the resolution of transmission is limited by the study setting. A PCR test for all family members on the same day as the student (and in the same laboratory) would have been ideal but was logistically not possible.

With regular and sensitive school pool PCR testing, infectious students can be identified early enough to avoid infection within the school setting, based on data from the B.1.1.7. variant. The outcomes and feasibility of this pilot study contributed to the decision of the Bavarian government to implement a PCR pool test concept for all Bavarian primary schools for the school year 2021/2022. However, as infections outside the schools are common and new variants increase the speed of transmission, school test systems cannot keep schools free of infections but can only slow down transmissions at best. In the end, testing cannot replace immunization.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/dmp.2022.279

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Conflict of interest. R.R. and D.W. are employed by NOVOGENIA, a commercial PCR test laboratory. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. **Kheiroddin P, Gründl M, Althammer M, *et al.*** How to implement safe, efficient and cost-effective SARS-CoV-2 testing in urban and rural schools within one month. *COVID*. 2021;1:717-727. doi: 10.3390/COVID1040057
2. **Scohy A, Anantharajah A, Bodéus M, *et al.*** Low performance of rapid antigen detection test as frontline testing for COVID-19 diagnosis. *J Clin Virol.* 2020;129:104455. doi: 10.1016/j.jcv.2020.104455
3. **Cibali E, Wenzel JJ, Gruber R, *et al.*** Pooling for SARS-CoV-2-testing: comparison of three commercially available RT-qPCR kits in an experimental approach. *Clin Chem Lab Med.* 2021;59(6):E243-E245. doi: 10.1515/CCLM-2020-1375
4. **Sweeney-Reed CM, Wolff D, Niggel J, *et al.*** Pool testing as a strategy for prevention of SARS-CoV-2 outbreaks in schools: protocol for a feasibility study. *JMIR Res Protoc.* 2021;10(5):e28673. doi: 10.2196/28673
5. **Kheiroddin P, Schöberl P, Althammer M, *et al.*** Results of WICOVIR Gargle Pool PCR testing in German schools based on the first 100,000 tests. *Front Pediatr.* 2021;9:1162. doi: 10.3389/FPED.2021.721518/BIBTEX
6. **Diederichs M, van Ewijk R, Isphording IE, *et al.*** Schools under mandatory testing can mitigate the spread of SARS-CoV-2. *Proc Natl Acad Sci U S A.* 2022;119(26):e2201724119. doi: 10.1073/PNAS.2201724119
7. **Paltiel AD, Zheng A, Walensky RP.** Assessment of SARS-CoV-2 screening strategies to permit the safe reopening of college campuses in the United States. *JAMA Netw Open.* 2020;3(7):e2016818. doi: 10.1001/JAMANETWORKOPEN.2020.16818
8. **Laub O, Leipold G, Toncheva AA, *et al.*** Symptoms, SARS-CoV-2 antibodies, and neutralization capacity in a cross sectional-population of German children. *Front Pediatr.* 2021;9:678937. doi: 10.3389/fped.2021.678937
9. **Yu J, Huang Y, Shen Z-J.** Optimizing and evaluating PCR-based pooled screening during COVID-19 pandemics. *Sci Rep.* 2021;11(1):21460. doi: 10.1038/s41598-021-01065-0
10. **Kheiroddin P, Gaertner VD, Schöberl P, *et al.*** Gargle pool PCR testing in a hospital during medium and high SARS-CoV-2 incidence. *J Hosp Infect.* 2022;127:69-76. doi: 10.1016/J.JHIN.2022.05.018