SARS-CoV-2 co-detection with influenza A and other respiratory viruses among school-aged children and their household members— March 12, 2020, to February 22, 2022, Dane County, Wisconsin

Jonathan L. Temte, MD/PhD[1]; Shari Barlow, BA[1]; Emily Temte, BA[1], Maureen Goss, MPH[1]; Allen Bateman, MPH/PhD[2]; Kelsey Florek, PhD[2]; Amra Uzicanin, MD/MPH[3]

[1]Department of Family Medicine and Community Health. University of Wisconsin School of Medicine and Public Health. Madison, WI

[2]Wisconsin State Laboratory of Hygiene. Madison, WI

[3]U.S. Centers for Disease Control and Prevention. Atlanta, GA

Corresponding author:

Maureen Goss, MPH

University of Wisconsin-Madison

Dept of Family Medicine and Community Health

1100 Delaplaine Court

Madison, WI 53715

Maureen.Landsverk@fammed.wisc.edu

Alternate corresponding author:

Jonathan L. Temte, MD/PhD

University of Wisconsin-Madison

Dept of Family Medicine and Community Health

1100 Delaplaine Court

Madison, WI 53515

jon.temte@fammed.wisc.edu

Running Title: SARS-CoV-2 and other virus co-detection

Abstract

Background: Concurrent detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and another respiratory virus in individuals can document contemporaneous circulation. We used an ongoing, community-based study of school-aged children and their households to evaluate SARS-CoV-2 co-detections with other respiratory viruses in a non-medically attended population over a two-year period.

Methods: Household enrollment was predicated on an acute respiratory illness in a child residing in that household who was also a kindergarten through 12th grade student in the participating school district. Demographic, symptom and household composition data, and self-collected nasal specimens were obtained on the recruitment day, and 7 and 14 days later, from the index child and all other household members. All specimens were tested for SARS-CoV-2/influenza A/B by RT-PCR. Day 0 specimens from the index children were simultaneously tested for 17 viruses using a commercial respiratory pathogen panel (RPP). To assess viral co-detections involving SARS-CoV-2, all household specimens were tested via RPP if the index child's Day 0 specimen tested positive to any of the 17 viral targets in RPP and any household member tested positive for SARS-CoV-2.

Results: Of 2,109 participants (497 index children in 497 households with 1,612 additional household members), two (0.1%) were positive for both SARS-CoV-2 and influenza A; an additional 11 (0.5%) were positive for SARS-CoV-2 and another RPP-covered respiratory virus. Co-detections predominantly affected school-aged children (12 out of 13 total) and were noted in 11 of 497 households.

Conclusions: SARS-CoV-2 co-detections with other respiratory viruses were uncommon and predominated in school-aged children.

Key Words: SARS-CoV-2, respiratory viruses, co-detection, children, households

Introduction: In Wisconsin, the first case of SARS-CoV-2 was reported on February 5, 2020 [1]. Subsequent statewide transmission largely mirrored national trends, from March 2020 to February 2022, with incidence culminating during January 2022 with a wave of the Omicron variant [2]. The emergence of SARS-CoV-2 was associated with a profound reduction in the circulation of other respiratory viruses both in Wisconsin [3] and across the United States [4].

Co-detection of other respiratory viruses with SARS-CoV-2 is defined as the concurrent detection of another viral pathogen [5,6]. Co-detections are of interest for four primary reasons: (1) co-detections can document other viruses in contemporaneous circulation leveraging the intensive specimen collection and testing for SARS-CoV-2 in the context of the ongoing COVID-19 pandemic, (2) presence and/or absence of co-detected viruses may inform us of potential viral competition and inhibition [7,8], (3) co-detections of SARS-CoV-2 with seasonal coronaviruses prompt the question of possible genetic recombination [9], and (4) coinfection could lead to an altered or worsened clinical syndrome for both adults and children [10].

Co-detections with SARS-CoV-2 have been uncommon according to assessments performed in clinical settings [11], although this may be driven by the reduced prevalence of other respiratory viruses and changes in testing patterns. When co-detections are noted, assessing the associated symptomology and epidemiological characteristics of cases could prove valuable, especially as other respiratory viruses return to greater circulation. We describe the types and estimated prevalence of SARS-CoV-2 co-detections within a Wisconsin community-based cohort of school-aged children and their household members over a continuous study period extending from March 12, 2020, to February 22, 2022, and we evaluate patterns of occurrence within households.

Methods: The ORegon CHild Absenteeism due to Respiratory Disease Study (ORCHARDS) is a longitudinal, school-based, community surveillance study evaluating the relationship between school absenteeism, community patterns of respiratory virus prevalence, and the levels of household transmission. The complete ORCHARDS protocol has been published elsewhere [12]. Enrollment is based upon the occurrence of an acute respiratory illness (ARI) in a kindergarten through 12th grade (K-12) student eligible to attend school in the Oregon School District (OSD; Dane County, WI). Participant included children enrolled in 4-year-old kindergarten programs. Dane County has a population of 546,000 and includes the Madison metropolitan area. On March 9, 2020, the ORCHARDS protocol was modified to provide safety for the research team and households following local detection of SARS-CoV-2; direct contact home visits were replaced by contactless drop-offs of household data collection forms and specimen collection kits. Eligibility criteria were expanded to include symptoms specific to COVID-19 (loss of taste or smell, nausea or vomiting) and suspected COVID-19 infection. Collection of symptom data and self-collected, anterior nasal specimens [13] from all household members occurred on the date of packet drop-off (Day 0) and seven days later (Day 7). Parents/guardians collected anterior nasal specimens from younger children. The protocol was again modified on October 6, 2020, at which time Day 14 symptom data and specimen collection were added allowing for a longer prospective observation interval for ascertainment of household transmission of SARS-CoV-2. This study was reviewed and approved by the University of Wisconsin Health Sciences Institutional Review Board.

Household data included the number of individuals residing in the household, their ages and roles, the number of bedrooms in the household, self-reported COVID-19 and influenza vaccine receipt status, and travel history within the week preceding symptom onset. Information on the illness episode (if applicable) included date of symptom onset, individual symptoms, and self-reported severity of

symptoms [12]. Severity was reported by a parent/guardian as 0=no symptoms; 1=mild; 2=moderate; 3=severe symptoms.

All self-collected specimens were tested within two days of specimen collection at the Wisconsin State Laboratory of Hygiene (WSLH) using a commercial multiplex assay kit based on reverse transcription polymerase chain reaction (RT-PCR) for detection of SARS-CoV-2, influenza A (FluA) and influenza B (FluB) [14]. In accordance with ORCHARDS protocol, all Day 0 specimens from the ORCHARDS index K-12 students were tested at the WSLH also within two days of specimen collection for 18 viruses (influenza [A, AH1, AH3, B], RSV [A, B], rhinovirus/enterovirus, parainfluenza virus [1, 2, 3, 4], human metapneumovirus, adenovirus, human coronavirus [HKU1, NL63, 229E, OC43], human bocavirus), and three bacteria (Chlamydophila pneumoniae, Mycoplasma pneumoniae, and Legionella pneumophila) using a commercial respiratory pathogen panel (RPP) [15]. For participating households in which SARS-CoV-2 was identified in any household member and the ORCHARDS index K-12 student tested positive for another virus on the routine Day 0 RPP, we tested all archived Day 0, 7, and 14 specimens from all household members via RPP. Cycle threshold (Ct) values were available for SARS-CoV-2, FluA and FluB results; Ct values were not available for the other viruses from RPP. Most of the SARS-CoV-2 variants were sequenced at WSLH using the Illumina MiSeq platform [16,17].

Age group (0-17 years; 18+ years) and severity (no symptoms, mild, moderate, severe) comparisons for individuals based on presence or absence of SARS-CoV-2 co-detection were performed using the chi-square test. Comparisons based on Ct values were performed using ANOVA. The study team also recorded when certain school mitigation measures were in place and later described infections over time relative to these preventive strategies.

Results:

ORCHARDS index children: From March 12, 2020, to February 22, 2022, ORCHARDS enrolled 497 index children meeting eligibility criteria (figure 1). Viruses were identified in 349 (70.2%) of self-collected Day 0 specimens. Rhinoviruses/enteroviruses (RE) were detected in 223 (44.9%), and SARS-CoV-2 was detected in 76 (15.3%) specimens. Respiratory syncytial virus type B (RSV-B) was detected in 14 (2.8%), human coronavirus OC43 (HCoV-OC43) in 13 (2.6%), FluA in 5 (1.0%), parainfluenza virus 3 (PIV-3) in 5 (1.0%); PIV-4 in 3 (0.6%), human metapneumovirus (hMPV) in 3 (0.6%), and HCoV-229E in 3 (0.6%) specimens. Four SARS-CoV-2 co-detections (0.8% [95% CI: 0.3—2.1%]) were noted in the Day 0 specimens from these 497 children. The prevalence of SARS-CoV-2 co-detection (n=4) in the subset of 76 index children with known SARS-CoV-2 was 5.3% [95%CI: 2.1—12.8].

Of note is the paucity of respiratory viruses identified from April through August 2020, during which in-person education was replaced with full-time distance learning in OSD (figure 2). Cessation of in-person education in the OSD started on Monday, March 16, 2020, and continued through August 2020. This was accompanied by an abrupt end to the influenza season in Wisconsin and very low prevalence of other respiratory viruses [3].

Starting at the beginning of the 2020-2021 academic year, limited in-person education was initiated, which continued through June 2021. This entailed the simultaneous presence of approximately 50% of students for half-day instruction supplemented by virtual education for the other half-day, four days a week, with a once-weekly distance learning day for the entire school. Universal mask wearing and physical distancing were enforced while in-person education occurred. Increases in respiratory viruses, especially RE, occurred throughout this period. Full-day, full cohort, in-person education, with enforced universal mask wearing, but without physical distancing resumed in September 2021. Concomitantly,

the prevalence and diversity of respiratory viruses increased as public health measures within the OSD were incrementally relaxed (figure 2). All co-detections occurred after August 2021, when a large spike of RE occurred at the resumption of in-person education.

Households: Over the same timeframe, we amassed 6,084 self-collected nasal specimens from 2,109 individuals in the 497 recruited households (including those from the 497 ORCHARDS index children; figure 1). Compliance of participating households was high with 96% of all possible specimens collected. RT-PCR results were positive for SARS-CoV-2 in 466 (7.7%) specimens, representing 273 (12.9%) individual cases, and positive for FluA in 19 (0.3%) specimens, representing 17 (0.8%) individual cases.

SARS-CoV-2 was detected in at least one member of 104 of the 497 households in the study (21%). Simultaneous testing using the RT-PCR assay for SARS-CoV-2/FluA/FluB revealed two cases of co-detection of SARS-CoV-2 and influenza A virus (0.4%) in December 2021, each in a separate household. Co-detections of SARS-CoV-2/FluA occurred in Day 7 specimens only, whereas SARS-CoV-2 was noted in all (i.e., Day 0, 7 and 14) specimens for both cases: one affected a 9-year-old, ORCHARDS index child vaccinated for both seasonal influenza and COVID-19, while the other affected a 46-year-old father from a different household who was vaccinated for COVID-19, but not for seasonal influenza. Both households had four members each (Table 1). Subtyping of the Influenza A virus was not possible for either of these two co-detections due to high Ct values (35.92 and 36.68, respectively; Table 1).

Per-protocol testing of the Day 0 specimens from the ORCHARDS index child was positive for another respiratory virus in 19 (3.8%) households (figure 1). Following our specimen selection algorithm, RPP testing was extended to the Day 7 and 14 specimens of the 19 ORCHARDS index cases as well to all additional household members and all specimens collected in these 19 households (i.e., to 70 additional household members) resulting in testing of 248 additional specimens. Of the 89 total individuals

residing in the 19 households, 33 (37%) were SARS-CoV-2 (+), while 51 (57%) were positive for another respiratory virus. SARS-CoV-2 co-detections with another RPP-covered virus were identified in seven additional individuals.

Overall, 13 co-detections were identified: four from the Day 0 testing of all ORCHARDS index children, two from SARS-CoV-2/Flu co-testing of all household specimens, and seven from the enhanced household testing. All 13 co-detections were noted during the 2021-22 school year, when the schools in the OSD opened for 100% in-person instruction. Sequential detections (SARS-CoV-2 preceded or followed by detection of another respiratory virus, but not with simultaneous detection) were noted in 6 (6%) individuals (Table 1; figure 1) within the 14-day sampling window.

Based on this enhanced-risk sample, the prevalence of SARS-CoV-2 co-detections in 21 households with known contemporaneous presence of both SARS-CoV-2 and another respiratory virus, and comprising 97 individuals, was estimated at 13.4% [95%CI: 8.0—21.6%]. The overall co-detection rate of 13 cases among all 2,109 individuals from 497 households participating in our study was 0.6% [95% CI: 0.3—1.1]. When restricted to the subset of 273 household members for whom SARS-CoV-2 was detected, the prevalence estimate for co-detection increased to 4.8% [95% CI:2.8—8.0]. Compared to the non-SARS-CoV-2 respiratory viruses circulating in school-aged children during the study period, HCoV-OC43 appeared to be overrepresented and RE appeared to be underrepresented in the sample of SARS-CoV-2 co-detection cases (Table 2).

The variant status of SARS-CoV-2 (Delta vs. Omicron) did not appear to be associated with co-detection. Four co-detections were with Delta. One additional co-detection occurred during the period of Delta circulation (November 2021 [2]) for which the SARS-CoV-2 virus was not sequenced. Six SARS-CoV-2 co-detections were with Omicron. Two additional co-detection specimens were not sequenced but

occurred during a time of Omicron predominance (January and February 2022 [2]). The Ct value of SARS-CoV-2 was not associated with the presence of a co-detected respiratory virus (ANOVA: P=0.392). The mean Ct value for SARS-CoV-2 in co-detection specimens was 26.6±6.0 as compared to Ct value of 28.2±6.3 for SARS-CoV-2 specimens without co-detection.

In 11 households with co-detections (two households had two co-detections each), there were 26 individuals with SARS-CoV-2, of which 13 (50%) had co-detections, among people aged from 7—46 years (Figure 3). Co-detections were significantly more likely to occur in children and adolescents (92%), with only one co-detection in an adult (chi-square = 10.085; P<0.001). The median age of individuals with co-detection was 10 years [mean: 14.3±10.1 (S.D.)] as compared to 40 years [mean: 34.5±14.8] for 13 household members with SARS-CoV-2 only. No differences in self-reported severity of illness were noted among individuals with SARS-CoV-2 co-detections (median = 2) as compared to household members with only SARS-CoV-2 detection (median = 1.5; chi-square: P=0.944).

Discussion:

Among K-12 school children (ages 4—18 years) and their household members with SARS-CoV-2 detection, we found that approximately 5% had co-detection of another respiratory virus. Despite high prevalence of SARS-CoV-2 among the 497 households studied (21%) and high prevalence of SARS-CoV-2 (15%) and other respiratory viruses within ORCHARDS index children (55%), the overall prevalence of SARS-CoV-2 co-detections among 4K-12 school children (ages 4—18 years) with ARI and/or COVID-19-like symptoms was <1%. Likewise, the prevalence among household members of the index children was also <1%. Whereas these findings were similar to the prevalence in SARS-CoV-2 co-detection in Brazil [11], our estimate is in stark contrast with other studies reporting co-detection rates in children as high as 51% [18-20]. Following the purposeful selection of individuals at enhanced risk for co-detection—

namely those residing in households with contemporaneously known SARS-CoV-2 and other respiratory virus cases—our estimate increased to 13.4%. The higher rates reported in other studies [18-21], however, emerged from clinical and hospital settings. The highest rate was reported for children admitted to a Wuhan hospital in January through February 2020, a time when other respiratory viruses were highly prevalent. The venue (i.e., community, clinic, hospital) and strategy for sampling play a significant role in SARS-CoV-2 co-detection, and influence the generalizability of co-detection prevalence estimates.

Detection of two SARS-CoV-2/FluA cases from two separate households was surprising, given the very low prevalence of influenza during the study period. These two cases, both in December 2021, coincided with the peak, albeit low, of FluA activity in Wisconsin [22]. Some authors have suggested that because seasonal coronaviruses and influenza viruses have similar molecular structures to SARS-CoV-2, coinfection with these pathogens may provide a protective, cross-reactive immune response [7,8]. If this is true, a recent infection with SARS-CoV-2 could lessen clinical consequences of a subsequent infection with an influenza virus via viral interference [8]; such a scenario would be consistent with the high Ct values noted for influenza A, which appeared seven days after initial SARS-CoV-2 detections for each SARS-CoV-2/FluA co-detection in this report. Further discussion of viral competition is beyond the scope of this study [23].

In this two-year study with continuous recruitment, we found SARS-CoV-2 co-detections reflected the level of circulation of other respiratory viruses among school-aged children. Other studies have shown a marked reduction in the circulation of viruses causing ARIs as a direct result of nonpharmaceutical interventions designed to mitigate the transmission of SRAS-CoV-2 [24]. Indeed, the prevalence of circulating respiratory viruses appears to inversely reflect the intensity of public health measures taken

by OSD schools in response to the SARS-CoV-2 pandemic. As shown in Figure 2, viral circulation apparently increased as physical distancing in schools decreased. Further scrutiny of this association merits additional study.

Although the low numbers of co-detections in this study preclude detailed analyses of the association of SARS-CoV-2 coinfection across variants, other respiratory viruses, vaccination status, household size, and travel history, we noted that RE and HCoV-OC43 appeared to be possibly underrepresented and overrepresented, respectively, as compared to their documented prevalence in Day 0 specimens taken from the index children in this study (Table 2). The relative frequency of SARS-CoV-2/HCoV-OC43 may be due to enhanced secondary transmission of HCoV-OC43 within households [25]. Coinfection with SARS-CoV-2 and seasonal coronaviruses theoretically could lead to genetic recombination, and thus may pose a potential public health threat [10] and has been recently shown for SARS-CoV-2 Delta and Omicron recombination [26]. Further surveillance and research of these co-detections would be helpful to evaluate their occurrence and implications. Our findings of lower-than-expected SARS-CoV-2/RE co-detection contrast with those from a study in Spain, which demonstrated persistence of SARS-CoV-2 in the presence of RE [27].

Co-detections were much more common in children (92%) than in adults (8%), in agreement with a similar household study from Switzerland [19]. Compared to that study, however, children with SARS-CoV-2-co-detections in our study were older (median age = 10 years vs. 4.2 years). The presence of another respiratory virus with SARS-CoV-2 did not result in significantly worse symptoms for individuals in our study based on self-report. This may be a function of the preponderance of respiratory virus cases within school-aged children [28] and the generally milder SARS-CoV-2 symptoms noted in children as compared to adults. [29]

There are several limitations and strengths in this study. First, the ORCHARDS study platform is child-centric. ORCHARDS enrolls K-12 school-aged children (ages 4-18 years) who have an acute illness consistent with an ARI or COVID-19 [12]. Adults and younger children (age 0—3 years) are recruited as part of a household, predicated on the presence of an illness episode in a school-aged child (index child) from the same household. Consequently, we will miss sampling from individuals with asymptomatic SARS-CoV-2, and from households without school-aged children (see: figure 3). Conversely, the sampling frame is efficient in that it enrolls individuals from the segment of the population with the highest prevalence of respiratory viruses [30]. Second, ORCHARDS is restricted to a single school district in south-central Wisconsin, and enhanced sampling for SARS-CoV-2 co-detections occurred during the initial 24 months of the COVID-19 pandemic (March 2020 – February 2022). Results from this study may not be generalizable to other populations or even the same population during a different period. Our study platform has, however, the benefit of being a long-standing investigation that has high visibility and acceptance by households comprising the OSD. There is high compliance with the study protocol [12] as evidenced by the 96% rate of specimen collection in this study. In addition, the community-based setting permits evaluation of a broader and more representative population compared to studies based in clinical or hospital settings. Third, we used self-collected anterior nasal specimens. This technique was used to allow non-contact sampling during the pandemic to both protect the study staff and to eliminate risk of staff-to-household transmissions. We have previously demonstrated high levels of specimen adequacy across a wide range of ages using self-collection [13], which enabled this transition from the very start of the pandemic. Fourth, we utilized self-reporting of symptom severity and depended on respondents' perceptions and recall. The inter-household validity of this reporting has not been assessed. Consequently, conclusions pertaining to effects of SARS-CoV-2 co-detection on severity should be regarded with caution. Fifth, we employed a sampling approach to laboratory testing

to maximize the identification of co-detections. We excluded households without SARS-CoV-2 detections and those in which the per-protocol Day 0 specimen of the ORCHARDS index case did not detect another respiratory virus. As a result, our prevalence estimate for co-detections within the household sample may be biased upward. Conversely, we would miss co-detection cases in households for which the index child did not have a detection of another respiratory virus. This is countered, however, by the consistency of our two estimates of prevalence: (1) four co-detections from 497 ORCHARDS index children (0.8%) who were routinely tested using SARS-CoV-2/FluA/FluB RT-PCR and RPP; and (2) 13 co-detections across all 2,109 participants, from whom a selected subset of specimens was tested using RPP (0.6%). Likewise, consistency existed across two prevalence estimates for SARS-CoV-2 co-detections for individuals with known SARS-CoV-2 detection: four co-detections from 76 ORCHARDS index children with SARS-CoV-2 (5.3%) vs. 13 co-detections among 273 household members with known SARS-CoV-2 detection (4.8%). Sixth, some co-detections may have been misclassified because of persistent shedding of SARS-CoV-2 RNA or other viruses from a previous infection in some participants. Our testing identifies nucleic acids from viruses and does not necessarily indicate infectivity. Finally, the number of detected SARS-CoV-2 co-detections with other respiratory viruses was too small to allow detailed analyses of possible factors contributing to co-detection, such as vaccination status, variant identified, household density, or travel history.

This longitudinal, community-based assessment of school-aged children and their household members identified a low rate of respiratory virus co-detection with SARS-CoV-2. Co-detections were far more common in children and reflected respiratory viruses in contemporaneous circulation in the study location as well as level of COVID-19 mitigation efforts in the school district. Future research should evaluate the frequency, diversity, and epidemiologic and clinical significance of SARS-CoV-2 co-detection

with other respiratory pathogens, the possibility of human coronavirus-SARS-CoV-2 recombination, the role of COVID-19 and/or influenza vaccination, and the role of viral interference.

NOTES

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FIGURE LEGENDS

Figure 1. Flow diagram of testing for ORCHARDS index cases (dashed arrows) and household selection for enhanced respiratory pathogen testing (solid arrows) and the final outcomes of testing among children and households. The entire cohort consists of 497 ORCHARDS child index cases and 1,612 additional household members. Testing was performed using first a combined RT-PCR (SARS-CoV-2/Flu/FluB) on Day 0, 7, and 14 specimens for each individual. All ORCHARDS index children were tested using a commercial respiratory pathogen panel (RPP) with 18 virus targets. Household members were also tested using RPP if SARS-CoV-2 was detected in the household and if the ORCHARDS child index case was positive for another respiratory virus. Bottom rows of boxes (heavy outlines) provide numbers of individuals based upon outcomes of testing.

Figure 2. SARS-CoV-2 (solid line) and other respiratory virus (stacked bars) detections in kindergarten through 12th grade students in the Oregon School District (Dane County, WI) by month of specimen collection, March 2020 through February 2022. Asterisks denote co-detections between the virus identified by the colored bar and SARS-CoV-2. (FluA = influenza A; FluB = influenza B; hMPV = human metapneumovirus; PIV = parainfluenza virus; NL63, OC43, and 229E are seasonal coronaviruses; RSVB = respiratory syncytial virus – type B; RE = rhinovirus/enterovirus). Mitigation strategies employed by the school district are provided within the arrows. The temporal trend in SARS-CoV-2 detections in Dane County, WI over this time period is available at: https://publichealthmdc.com/coronavirus/dashboard.

Figure 3. Age distribution of individuals with SARS-CoV-2 (SC2) co-detection with another respiratory virus and individuals (blue bars) with a SC2 detection (green bars) only from 11 households with identified SC2 co-detections—Dane County, Wisconsin, March 2020 to February 2022. The age axis is split due to no individuals identified between the ages of 20 and 35 years.

EPIDEMIOLOGIC INFORMATION BY HOUSEHOLD AND PARTICIPANT						LABORATORY RESULTS BY DAY OF SAMPLE COLLECTION (NUMBER REFLECTS DAYS FROM THE ENROLMENT DATE OF THE ORCHARDS CHILD)								
						Day 0			Day 7			Day 14		
Households and Participants (age in years)	Received seasonal influenza vaccine?*	Received a COVID-19 vaccine 14+ days before Day 0?* *ND = No dates*	Recent travel in week prior to onset	Symptom severity **	Symptom Onset Relative to Day 0	SARS-CoV-2 (Ct value)	Influenza A	Multiplex*	SARS-CoV-2 (Ct value)	Influenza A (Ct value)	Multiplex*	SARS-CoV-2 (Ct value)	Influenza A	Multiplex*
Household 1 (5 bedrooms)						**9/19/2021**			**9/26/2021**			**10/3/2021**		
ORCHARDS Child (17)	No	Yes	No	Mild	**Day -3**	**Positive, δ (20.16)**	Negative	**Rhino/ Enterovirus**	**Positive, δ (23.91)**	Negative	Negative	Negative	Negative	Negative
Mother (44)	No	Yes		N/A	N/A	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Father (50)	No	Yes	No	Mild	**Day -7**	**Positive, δ (21.99)**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Sibling 1 (15)	No	Yes	No	Mild	**Day -2**	Negative	Negative	**Rhino/ Enterovirus**	Negative	Negative	**Rhino/ Enterovirus**	Negative	Negative	Negative
Sibling 2 (11)	No	No	No	Mild	**Day 7**	Negative	Negative	Negative	Negative	Negative	**Rhino/ Enterovirus**	Negative	Negative	Negative
Household 2 (4 bedrooms)						**11/11/2021**			**11/18/2021**			**11/25/2021**		
ORCHARDS Child (12)	Yes	Yes	No	Moderate	**Day -1**	Negative	Negative	**Rhino/ Enterovirus**	Negative	Negative	Negative	Negative	Negative	Negative
Father (40)	Yes	Yes	No	Moderate	**Day -13**	**Positive (33.01)**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	**Rhino/ Enterovirus**
Mother (40)	Yes	Yes		N/A	N/A	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Sibling (10)	Yes	No	No	Mild	**Day -17**	Negative	Negative	Negative	**Positive (34.33)**	Negative	**Rhino/ Enterovirus**	Negative	Negative	Negative
Household 3 (3 bedrooms)						**11/30/2021**			**12/7/2021**			**12/14/2021**		
ORCHARDS Child (9)	Yes	Yes (1 dose)	Yes	Mild	**Day -1**	**Positive, δ (17.05)**	Negative	**Rhino/ Enterovirus**	**Positive (26.54)**	Negative	**Rhino/ Enterovirus**	Negative	Negative	**Rhino/ Enterovirus**
Mother (40)	Yes	Yes	No	Mild	**Day 3**	Negative	Negative	Negative	**Positive (36.16)**	Negative	Negative	Negative	Negative	Negative
Father (43)	Yes	Yes		N/A	N/A	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Sibling (11)	Yes	Yes (1 dose)	Yes	Mild	**Day -2**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Household 4 (4 bedrooms)						**12/6/2021**			**12/13/2021**			**12/20/2021**		

ORCHARDS Child (9)	Yes	No	No	Mild	**Day -3**	**Positive, δ (16.42)**	Negative	Negative	**Positive (31.49)**	**Positive (35.92)**		**Positive (35.82)**	Negative	
Mother (42)	Yes	Yes		N/A	N/A	**Positive (33.55)**	Negative		Negative	Negative		Negative	Negative	
Father (45)	Yes	Yes	No	Moderate	**Day -8**	**Positive, δ (25.19)**	Negative		**Positive (35.52)**	Negative (41.03)		**Positive (33.05)**	Negative	
Sibling (12)	Yes	Yes		N/A	N/A	Negative	Negative		Negative	Negative		Negative	Negative	
Household 5 (4 bedrooms)						**12/16/2021**			**12/23/2021**			**12/30/2021**		
ORCHARDS Child (12)	Yes	No	No	Moderate	**Day -13**	Negative	Negative	Negative	Negative	Negative		Negative	Negative	
Mother (42)	No	Yes	No	Mild	**Day -11**	**Positive, δ (16.16)**	Negative		**Positive (35.62)**	Negative		Negative	Negative	
Father (46)	No	Yes	No	Moderate	**Day -13**	**Positive, δ (22.79)**	Negative		**Positive, δ (29.06)**	**Positive (36.68)**		**Positive (36.90)**	Negative	
Sibling (7)	Yes	No	No	Moderate	**Day -20**	Negative	Negative		Negative	Negative		Negative	Negative	
Household 6 (3 bedrooms)						**12/31/2021**			**1/7/2022**			**1/14/2022**		
ORCHARDS Child (12)	Yes	Yes	Yes	Severe	**Day -3**	**Positive, o (19.23)**	Negative	**HCoV-OC43**	**Positive, o (22.48)**	Negative	**HCoV-OC43**	Negative	Negative	Negative
Mother (43)	Yes	Yes	Yes	Mild	**Day 3**	Negative	Negative	Negative	**Positive, o (25.07)**	Negative	Negative	**Positive, o (27.11)**	Negative	Negative
Father (45)	Yes	Yes	Yes	Severe	**Day -2**	Negative	Negative	**HCoV-OC43**	Negative	Negative	Negative	Negative	Negative	Negative
Sibling 1 (12)	Yes	Yes	Yes	Severe	**Day -4**	Negative	Negative	**HCoV-OC43**	**Positive (35.31)**	Negative	Negative	**Positive, o (28.06)**	Negative	Negative
Sibling 2 (9)	Yes	Yes	Yes	Severe	**Day -1**	Negative	Negative	**HCoV-OC43**	**Positive, o (25.64)**	Negative	**HCoV-OC43**	**Positive (27.29)**	Negative	Negative
Household 7 (3 bedrooms)						**1/12/2022**			**1/19/2022**			**1/26/2022**		
ORCHARDS Child (7)	No	No	No	Moderate	**Day -2**	Negative	Negative	**Rhino/ Enterovirus**	**Positive (30.79)**	Negative	**PIV 4**	**Positive, o (21.62)**	Negative	Negative
Mother (37)	No	Yes	No	Moderate	**Day -1**	**Positive (37.2)**	Negative	Negative	**Positive, o (25.2)**	Negative	Negative	Negative	Negative	Negative
Father (36)	No	No	No	Mild	**Day -2**	**Positive, o (23.05)**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	**PIV 4**
Sibling 1 (16)	No	Yes (ND)	No	Moderate	**Day -2**	Negative	Negative	Negative	**Positive (33.95)**	Negative	**hMPV**	**Positive, o (22.51)**	Negative	Negative
Sibling 2 (5)	No	No	No	Mild	**Day -1**	Negative	Negative	**Rhino/ Enterovirus**	Negative	Negative	**PIV 4**	**Positive, o (28.84)**	Negative	Negative
Household 8						**1/20/2022**			**1/27/2022**			**2/3/2022**		

(5 bedrooms)														
ORCHARDS Child (7)	Yes	Yes	No	Moderate	**Day -2**	Negative	Negative	**Rhino/ Enterovirus**	Negative	Negative	**Rhino/ Enterovirus**	Negative	Negative	Negative
Mother (39)	Yes	Yes (ND)		N/A	N/A	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Father (45)	Unknown	Yes		N/A	N/A	No swab collected			No swab collected			Negative	Negative	Negative
Sibling 1 (16)	Yes	Yes	Yes	Moderate	**Day -4**	**Positive, o (17.77)**	Negative	Negative	**Positive (29.45)**	Negative	Negative	**Positive (28.46)**	Negative	**HCoV-OC43**
Sibling 2 (5)	No	Yes		N/A	N/A	Negative	Negative	Negative	Negative	Negative	**Rhino/ Enterovirus**	Negative	Negative	**Rhino/Enterovirus**
Sibling 3 (3)	Unknown	Unknown				No swab collected			No swab collected			No swab collected		
Household 9 (5 bedrooms)						**1/21/2022**			**1/28/2022**			**2/4/2022**		
ORCHARDS Child (4)	Yes	No	No	Moderate	**Day -1**	Negative	Negative	**RSV B**	Negative	Negative	**RSV B**	Negative	Negative	**RSV B**
Mother (37)	Yes	Yes	No	Mild		Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Father (36)	Yes	Yes	No	Mild	**Day -2**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Sibling 1 (17)	Yes	Yes	No	Mild	**Day 8**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	**RSV B**
Sibling 2 (14)	Yes	Yes		N/A	N/A	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Sibling 3 (10)	Yes	Yes		N/A	N/A	Negative	Negative	**RSV B**	**Positive (35.47)**	Negative	**RSV B**	Negative	Negative	Negative
Sibling 4 (<1)	No	No	No	Severe	**Day -29**	Negative	Negative	**RSV B**	Negative	Negative	**hMPV**	Negative	Negative	**RSV B, hMPV**
Household 10 (4 bedrooms)						**1/24/2022**			**1/31/2022**			**2/7/2022**		
ORCHARDS Child (12)	Yes	Yes (ND)	No	Moderate	**Day -4**	Negative	Negative	**HCoV-OC43**	**Positive (35.4)**	Negative	Negative	**Positive, o (25.47)**	Negative	Negative
Father (42)	Yes	Yes (ND)	No	Moderate	**Day 0**	Negative	Negative	Negative	Negative	Negative	**HCoV-OC43**	Negative	Negative	**HCoV-OC43**
Mother (40)	No	Yes (ND)	Yes	Severe	**Day -1**	**Positive (30.84)**	Negative	Negative	**Positive, o (24.84)**	Negative	Negative	Negative	Negative	Negative
Sibling 1 (10)	Yes	Yes (ND)		Severe	**Day 6**	Negative	Negative	Negative	**Positive, o (19.57)**	Negative	**HCoV-OC43**	**Positive (32.65)**	Negative	Negative
Sibling 2 (8)	Yes	Yes (ND)	No	Moderate	**Day -2**	Negative	Negative	Negative	Negative	Negative	**HCoV-OC43, Adenovirus**	Negative	Negative	**hMPV, Adenovirus**
Sibling 3 (6)	Unknown	No	No	Moderate	**Day -9**	Negative	Negative	**HCoV-OC43**	Negative	Negative	**hMPV**	Negative	Negative	Negative
Sibling 4 (5)	Unknown	No	No	Moderate	**Day -9**	Negative	Negative	**hMPV**	Negative	Negative	**hMPV**	Negative	Negative	Negative

Household 11 (4 bedrooms)						2/7/2022			2/14/2022			2/21/2022		
ORCHARDS Child (15)	Yes	Yes	No	Moderate	**Day -3**	**Positive (24.3)**	Negative	**HCoV-229E**	Negative	Negative	Negative	Negative	Negative	Negative
Mother (50)	Yes	Yes	No	Moderate	**Day 1**	Negative	Negative	Negative	Negative	Negative	**HCoV-229E**	Negative	Negative	Negative
Father (50)	Yes	Yes	No	Moderate	**Day 1**	Negative	Negative	**HCoV-229E**	Negative	Negative	**HCoV-229E**	Negative	Negative	Negative
Sibling 1 (20)	Yes	Yes	No	Mild	**Day -2**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Sibling 2 (17)	Yes	Yes	No	Mild	**Day 3**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

Table 1: Households with study participants who experienced codetection of SARS-CoV-2 with influenza A virus (N=2) or another respiratory virus (N=11) while participating in the ORegon CHild Absenteeism due to Respiratory Disease Study (ORCHARDS), Dane County, Wisconsin, March 12, 2020, to February 22, 2022. Red boxes indicate SARS-CoV-2 codetections. Blue boxes indicate three addition codetections of other respiratory viruses. Results of SARS-CoV-2 strain sequencing are given for specimens as available (δ = Delta variant, o = Omicron variant).

* Vaccination history as reported by participants or parents/guardians. Type(s) of vaccines were not ascertained.

** Illness severity as perceived and reported by the participant or participant's parent/guardian.

*** Multiplex testing is performed using a commercial multiple PCR respiratory pathogen panel (Luminex. NxTAG Respiratory Pathogen Panel) with 17 targets (influenza [A, AH1, AH3, B], RSV [A, B], rhinovirus/enterovirus, parainfluenza virus [1, 2, 3, 4], human metapneumovirus, adenovirus, coronavirus [HKU1, NL63, 229E, OC43], human bocavirus). Multiplex testing was routinely performed for the index ORCHARDS child on Day 0; other household members were tested by the same multiplex panel only if the index ORCHARDS child was positive on any of the targets. No Ct values are available for multiplex results.

Table 2. Number (percentages) of detections of non-SARS-CoV-2 (SC2) respiratory viruses. Data are presented for school-aged children (ORCHARDS index children; n=497) and all household members (including ORCHARDS index children) with SC2-codetection (n=13). ORegon CHild Absenteeism due to Respiratory Infection Study (ORCHARDS) (Dane County, Wisconsin: March 12, 2020 —February 22, 2022).

	HCoV-229E	HCoV-OC43	hMPV	FluA	PIV-4	RSVB	RE
ORCHARDS Index Children (n=497)* (% of children)	3 (0.6)	13 (2.6)	3 (0.6)	5 (1.0)	3 (0.6)	14 (2.8)	223 (44.9)
Individuals with SC2-codetections (n=13) (% of individuals)	1 (7.7)	4 (30.8)	1 (7.7)	2** (15.4)	1 (7.7)	1 (7.7)	3 (23.1)

Data are displayed for seasonal human coronaviruses (HCoV), human metapneumovirus (hMPV) influenza (Flu), parainfluenza virus (PIV), respiratory syncytial virus (RSV) and rhinovirus/enterovirus (RE).

* Number of detections from Day 0 specimens obtained from enrolled ORCHARDS index children during the study period (n=497). This does not include cases with HCoV-NL63 (n=6), influenza B (n=3), PIV-2 (N=2), and PIV-3 (n=5) as there were no SC2 codetections with these viruses within households.

** Includes one adult case. All other SC2 codetections were in children ages 7—17 years.

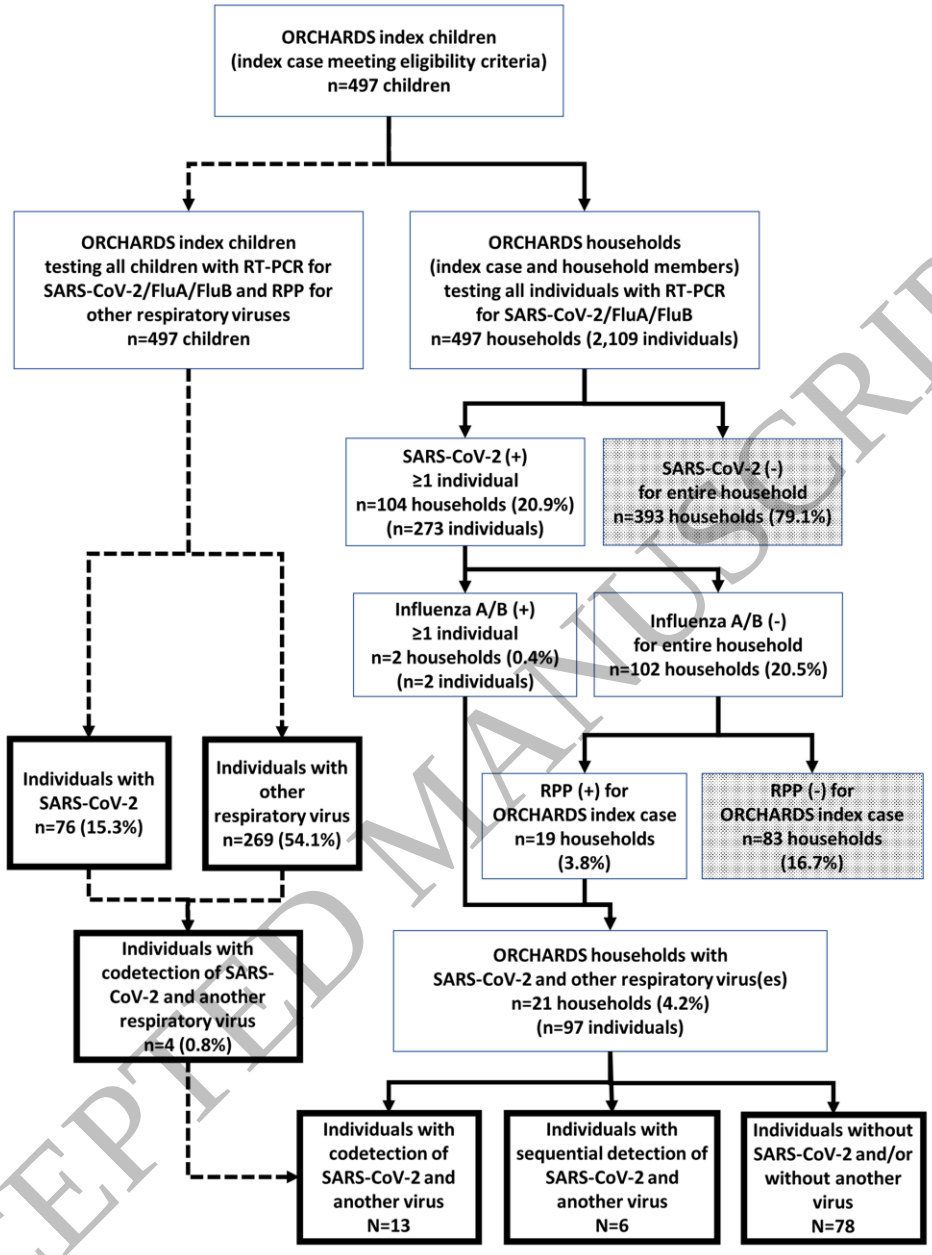

Figure 1
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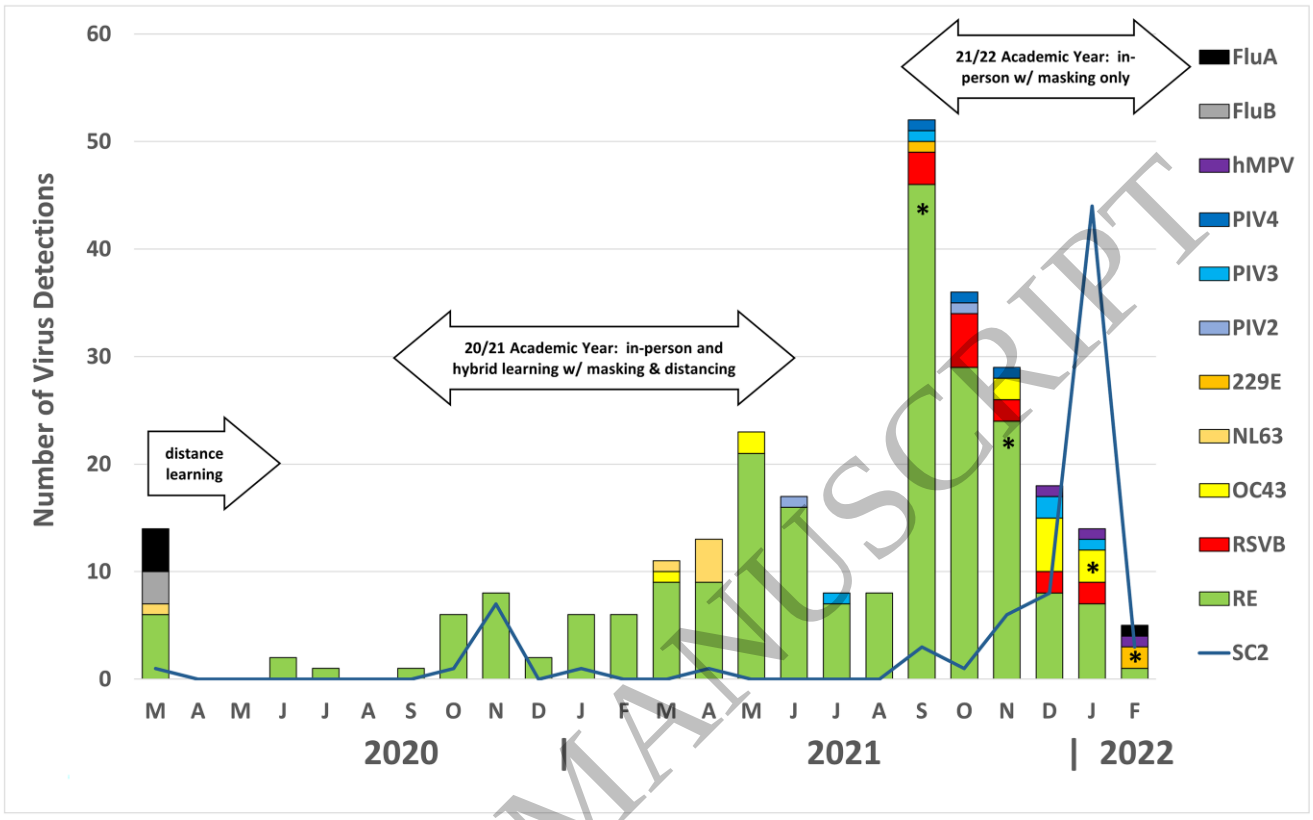

Figure 2
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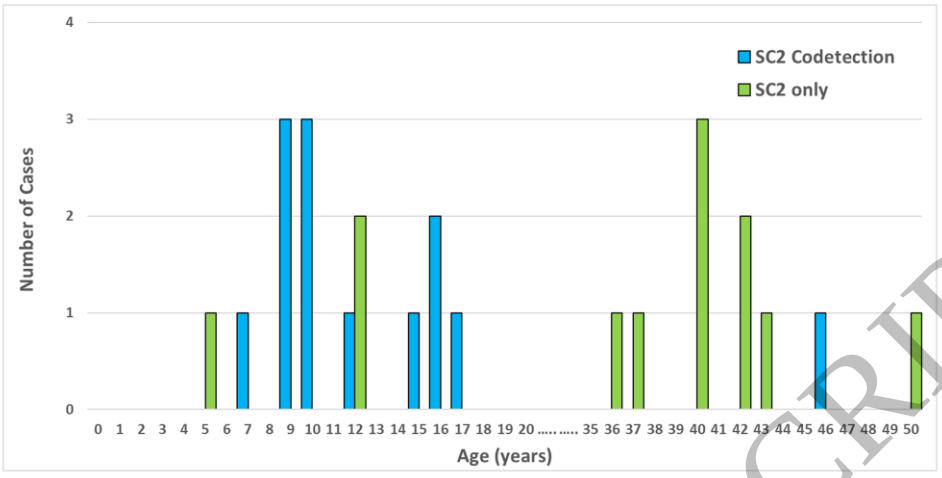

Figure 3
127x65 mm (x DPI)