Transmission of SARS-CoV-2 by children to contacts in schools and households: a prospective cohort and environmental sampling study in London

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Abstract

Background Assessing transmission of SARS-CoV-2 by children in schools is of critical importance to inform public health action. We assessed frequency of acquisition of SARS-CoV-2 by contacts of children with COVID-19 in schools and households, as well as the amount of virus shed into the air and onto fomites in both settings.

Methods Cases of COVID-19 in children in London schools were identified via notification. Weekly sampling for 3-4 weeks and PCR testing for SARS-CoV-2 of immediate classroom contacts (the “bubble”), non-bubble school contacts, and household contacts was undertaken supported by genome sequencing, along with surface and air sampling in the school and home environment.

Results. Within schools, secondary transmission was not detected in 28 individual bubble contacts, representing 10 distinct bubble classes. Across 8 non-bubble classes, 3/62 pupils tested positive– all three were asymptomatic and tested positive in one setting on the same day, unrelated to the original index case. In contrast, the secondary attack rate in naïve household contacts was 14.3% (5/35) rising to 19.1% (9/47) when considering all household contacts. Environmental contamination with SARS-CoV-2 was rare in schools, regardless of school type; fomite SARS-CoV-2 RNA was identified in 4/189 (2.1%) samples in bubble classrooms, 2/127 (1.6%) samples in non-bubble classrooms, and 5/130 (3.8%) samples in washrooms. This contrasted with fomites in households, where SARS-CoV-2 RNA was identified in 60/248 (24.2%) bedroom samples, 66/241 (27.4%) communal room samples, and 21/188 (11.2%) bathroom samples. Air sampling identified SARS-CoV-2 RNA in just 1/68 (1.5%) of school air samples, compared with 21/85 (24.7%) of air samples taken in homes.

Summary The low levels of environmental contamination in schools are consistent with low transmission frequency and adequate levels of cleaning and ventilation in schools during the period of study. Secondary transmission in schools was rare. The high frequency of secondary transmission in households associated with evident viral shedding throughout the home suggests a need to improve advice to households with infection in children in order to prevent onward community spread by sibling and adult contacts. The data highlight that transmission from children is very likely to occur when precautions are reduced.

Introduction.

The potential for schools to amplify outbreaks is well-recognised (1-3). School closures were associated with a reduction in COVID-19 incidence and mortality at the start of the pandemic (4,5) albeit effects remain confounded by other non-pharmaceutical interventions. Importantly, any benefits of closures must be weighed against the unquestionable harms to children and wider society.

COVID-19 poses a much lower risk to children than to adults, both in terms of illness severity (6) and risk of acquisition; children appear half as likely as adults to acquire SARS-CoV-2 (7). The onward transmission risk from SARS-CoV-2-infected children has been subject to less rigorous evaluation, though shedding of virus by children is not markedly different to adults (8). Point prevalence studies indicating a low prevalence of SARS-CoV-2 in schools (9) makes large scale monitoring of transmission impractical and uneconomic. Although outbreaks provide an opportunity to study onward transmission, these are often complicated by uncertainty about timing and transmission direction; one study in schools did identify children as a source of onward transmission in a small number of cases, but such outbreaks comprised just two cases on average (10).

Forward contact tracing offers an opportunity to search actively for secondary infections in a controlled manner. Despite this, transmission of respiratory infection in schools is seldom quantified except in the context of major outbreaks. Clinical attack rates of 20-30% are reported in schools affected by influenza A (11), however the role of silent infection and onward transmission from such cases is not well-studied. In a scarlet fever contact tracing study, we found that outbreak strains spread to over one-quarter of classroom contacts, despite treatment and isolation of index cases. (12) The potential for classrooms and asymptomatic 'shedders' to act as an accelerator for respiratory infection is therefore undeniable.

We adapted our contact-tracing protocol to investigate transmission of SARS-COV-2 by children in schools and households. The TraCK (Transmission of Coronavirus-19 in Kids, ISRCTN 13773960) study aimed to assess the risk posed by a SARS-CoV2-infected child who attends school, via longitudinal sampling of the child, school and household contacts, and associated environments, to evaluate and inform interventions to limit spread of COVID-19.

Methods

Study eligibility Schools in London reporting new cases of SARS-CoV-2 infection to local Health Protection teams were invited to take part if a child (index case) had been attending school in the 48h prior to a positive PCR test for SARS-CoV-2. Contextual information relating to prevailing regulations are in Appendix p8. Parents/guardians of notified cases were invited to allow their child and wider household to participate in the study. If the school was willing to support the study, parents/guardians

of contacts were also invited to allow their child to participate in the study. The study commenced October 9th 2020 and recruitment ended July 18th 2021.

Case definition. Children aged 2-14 years (extended to <18 years in November 2020) with a new nose and/or throat swab PCR test result positive for SARS-CoV-2 from an accredited laboratory. Findings from cases will be reported elsewhere.

Contact definition. Bubble contacts (BC) were children identified by schools who were required to isolate at home due to direct contact with a case. Non-bubble school contacts (SC) were children from a different 'control' class in the same school. Household contacts (HC) were adults and children of any age normally resident with the case, and required to isolate.

Contact sampling Combined nose and throat samples (single swab of throat followed by nostrils) were taken by the research team from each participating contact (BC, SC, or HC) as soon as possible (<48 hours) after case identification, and thereafter weekly for a total of 4 visits (3 visits from December 2020).

Environmental sampling. In households, surface and air samples were obtained in each of three rooms (child's bedroom, communal room, bathroom) at the first visit and thereafter weekly for a minimum of 4 visits (3 visits from December 2020); in some households sampling was undertaken more frequently in the first two weeks. In schools, surface and air samples were obtained weekly from the bubble classroom, school contact classroom, and washrooms. (for details see Appendix p9-10)

Virological testing. Nasopharyngeal swabs were tested for SARS-CoV-2 E-gene RNA and human RNAseP RNA by an accredited, quantitative RT-PCR followed by genome sequencing (Appendix p9-10). (13). Results were reported in real-time to participants and positive results subject to statutory reporting and associated regulations. Environmental samples were tested by a research laboratory (14) (Appendix p10).

Gingival crevicular fluid (GCF). GCF samples were collected from contacts on each sampling occasion (Appendix p11) then tested for total IgG against SARS-CoV2 nucleoprotein by the reference laboratory (15).

*Ethical approval***.** The study was approved by a research ethics committee (Schools Transmission Study REC reference 18/LO/0025; IRAS Reference 225006). Written, informed consent was obtained from all participants or parents/guardians, and assent was obtained from participants aged under 18.

Statistical analysis. Analysis was mostly descriptive due to sample size (Appendix p9); Fisher's exact test was used to compare proportions of household contacts with positive results (Stata version 15). Human target RNAs were compared using Mann Whitney U test (GraphPad Prism).

Role of the Funding Source None

Results.

Eight schools participated, of which 5 were primary, 2 secondary, and 1 was a special educational needs (SEND) school. In the course of the study, 428 combined nose and throat swabs and GCF samples were obtained from contacts of index cases. Environmental sampling included a total of 1620 surface samples, of which 446 were from schools, and 218 air samples, of which 68 were from schools.

Transmission to Bubble Contacts.

BC were recruited from 10 bubbles in 8 schools. In total 28 bubble contacts who were required to quarantine at home, were followed weekly. Onward transmission of SARS-CoV-2 to the 28 participating BC was not detected over the sampling period (Figure 1A, Table 1). Only 4/28 (14.3%) BC had evidence of prior exposure to SARS-CoV-2 from GCF testing. In one setting, a non-participating BC developed a fever and reported a positive community test. That child was recruited as a 'case' along with their household. Subsequent study sample PCR tests were negative, but GCF seroconversion at 4 weeks was consistent with this child being a co-primary case in the class. Participation rate among BC in each school varied widely (median 8.5%, range 2.4% - 26.9%), being lowest in SEND and secondary schools.

Transmission to and between non-bubble School Contacts.

Sixty-two pupil SC and 3 staff were recruited from the same 8 schools. SC participation rates were higher than BC, median 22.4% (range 5.2-54.5%). Of those tested, 13/65 (20%) had GCF antibodies indicating previous SARS-CoV-2 infection.

In 7/8 participating schools, no SC were found to be infected with SARS-CoV-2. In setting E, a secondary school, all SC tested negative in week 1, but in week 2, unexpectedly, SARS-CoV-2 was detected in swabs of 3/10 SC. (Figure 1B, Table 2). All three were asymptomatic; in one, the viral load increased from 293,240 E gene copies/swab to 5,999,560/swab copies 3 days later and onward transmission to a sibling household contact who shared a bedroom (84,040 E gene copies/swab) was observed. The other two asymptomatic SC had very low viral loads; the first had 280 E gene copies/swab but was tested only once. The second had 560 E gene copies/swab; samples 7 days earlier, and 4 days later were PCR-negative; and anti-SARS-CoV-2 antibodies were already present in GCF in weeks 1 and 2. It was felt possible that these low viral levels did not represent true infections, but transient mucosal contamination while in the company of a fellow pupil with active infection. The original index case in setting E had been identified following a community PCR test; by week 1 of SC testing the index case had a negative PCR test and was still quarantined. It was inferred that the infection in SC was not linked directly to the original index case.

Transmission to Household contacts.

Sixteen households took part, comprising 47 HC and 16 index cases who were each an index or co-primary case to a bubble class. The number of households exceeded the number of bubbles that participated because, in four settings, HC agreed to take part, but the relevant schools withdrew. In one setting, the school agreed to take part, but the HC withdrew; a separate case from the same class was identified by community testing however, and their HC were recruited. For setting E, HC of two of the three newly identified SC infections were included. All of the index cases were symptomatic except these two.

Of the HC, 3 children and 9 adults were already reported to be infected at the start of sampling. Initial analysis focussed on HC who were considered naïve (n=35) i.e. were not reported to be infected at the start of sampling, of which 11/35 were children. (Table 3)

Over the sampling period, 9 new infections were detected among naïve HC in 8 adults and 1 child (Table 3, Figure 1C). In two households, genome sequencing revealed that the index case was unrelated to the new adult HC infections (2 per household), hence these represented secondary introduction from the community (Table 3, Appendix p5). In all other households genome sequencing was consistent with clonal household transmission (Appendix p5). Transmission by children therefore resulted in infection of 5/35 (14.3%) naïve HC. Only 1/35 (2.9%) GCF samples suggested prior COVID-19 exposure among naïve HC at the start of sampling though this rose to 6/26 (23.1%) by the end of sampling (p=0.035). Just 6 HC had been partially or fully-vaccinated; these were 2 adults each in settings K1, K2, and M.

Twelve HC who were reported to be already-infected prior to study team arrival were also sampled sequentially, but were not included in the initial analysis, due to uncertainty of transmission direction. To gain greater insight into the frequency of secondary attack rate, symptom and testing history were reviewed. Three child HC were reported to be positive prior to research sampling; based on symptom onset and date of testing, it was deduced that these child HC had been secondarily infected by the index pupil in the home. Nine adults (from 5 households) were reported to be positive prior to research sampling. For 5/9 adults, test results and/or symptoms pre-dated that of the index child, suggesting that the child was not the index case within the household. For 4/9 adults, their infection was believed to arise from the index child. Taking these additional cases into consideration, the 16 index children resulted in 9 new cases in 47 household contacts (19.1% secondary attack rate).

Environmental samples in schools

Surface sampling identified SARS-CoV-2 in only 4/189 (2.1%) samples from bubble classrooms; 2/127 (1.6%) samples in school contact classrooms, and 5/130 (3.8%) samples from school bathrooms. (Figure 2A-C). Where detected, viral copy numbers were at the lower limits of detection except the edge of an index child's chair in a bubble classroom that had $>10^4$ E gene copies per swab in week 1, prior to deep cleaning. The same items were sampled in each location on a weekly basis (Appendix p2-3); no item became positive on subsequent sampling. Air sampling was undertaken

weekly in bubble classrooms, control classrooms, and washrooms, as soon as possible after children vacated those rooms, except when availability of equipment components limited this. Only 1/68 (1.5%) air samples was positive: This was at the limit of detection, in week 2 in a school that had experienced a number of staff infections, but in a control SC classroom not known to have any pupil COVID-19 cases.

We considered the possibility that air samples might only be positive when a room is in active use. To provide context, we undertook environmental sampling in a university building (appendix p4). We identified SARS-CoV-2 in 3/10 surface samples from a small office 4 days after use by a confirmed case of COVID-19, but not in any other office or location in the same building, or on follow up (0/96 samples). We also detected low levels of SARS-CoV-2 in an air sample from the same office 4 days after use; all air samples were negative when re-tested two weeks later (Appendix p4).

Environmental samples in households

In contrast to findings in schools, overall 262/1174 (22.3%) surface samples were found to be contaminated with SARS-CoV-2 in 16 households. Focussing on samples taken on the first visit and thereafter weekly, there was a trend to declining virus detection over time (Figure 2 D-F). The most frequent surface contamination was identified in index case bedrooms, where 60/248 (24.2%) samples tested positive, and communal rooms, where 66/241 (27.4%) samples tested positive. In bathrooms, 21/188 (11.2%) surface samples tested positive, consistent with increased bathroom surface cleaning. Personal items relating to the child such as pillows, and digital equipment such as mobile phones, remote controls and digital toys were more persistently positive over the sampling period whereas other sample types became negative within 2-3 weeks, including pet fur (Appendix p6). Surface human RNA levels were higher in households than schools (Appendix p7).

Overall, 42/150 (28%) air samples obtained in households were contaminated with SARS-CoV-2. Focussing on samples taken on the first visit and thereafter weekly, air samples were positive in 4/22 (18.2%) samples taken in the index child's bedroom; 13/42 (30.9%) samples in the communal room; and 4/21 (19%) samples in the bathroom (Figure 2D-F). Virus levels in air were highest in the room with an infected child and infected adults. The index child and household contacts were always in the communal (living) room at the time of sampling except three settings where the index child was only in the bedroom during sampling, and one setting where the child moved between rooms. There was no apparent association between the type of dwelling (apartment or house) and air contamination. Air samples in households and schools did not differ significantly with regard to human RNA (Appendix p7).

Discussion

Conducted during a period of enhanced precautions, transmission from index pupils to bubble contacts, and to other pupils in the school who were not close contacts, was not actively detected. Although the study was small, the findings contrasted with a secondary attack rate of at least 14.3%

in household contacts of the same index cases. When household contacts who had already been tested were included in our analysis, the secondary attack rate in households with a child index case was 19.1%.

One apparent transmission incident in a class that were not isolating involved three asymptomatic pupils, who could not be linked to the original index case in that school. One of these pupils had a high viral load, leading to detection of a secondary case in a household contact and, we believe, accounted for transient low viral loads detected in two other pupils. The low viral loads were similar to environmental samples and may be consistent with transient carriage on mucosae rather than early or late infection.

Environmental surface and air sampling was conducted to understand mechanisms of transmission, where transmission occurred. This showed little or no contamination in schools including surfaces touched frequently by children, providing a high level of reassurance regarding the school environment during a period of enhanced vigilance, underlined by a difference in human RNA detection between surfaces in households and schools. This contrasted with repeated identification of virus on household items frequently touched by children, and in the air around the home, particularly where the child was present. This is perhaps not surprising since the dimensions of domestic rooms are ~4 times smaller than classrooms and provides some insight into the risks of virus acquisition in the two settings. The detailed environmental sampling identifies digital equipment and personal items as potential fomite vectors, or as metrics of infectivity. The high proportion of air samples that were positive in the home compared with school underlines the greater risks associated with smaller rooms and is a reminder that air may remain positive for some time if not well ventilated. We considered the possibility that air sampling in schools was negative because the children were not present in the room, however control human RNA was no different in the air between schools and households. Control sampling in a different educational setting demonstrated low levels of SARS-CoV-2 RNA in the air 4 days after an office was used by a staff member with COVID-19. The low or absent levels of SARS-CoV-2 RNA in the bubble classroom also provides reassurance about the potential for ongoing infection in members of the bubble who returned to school by week 2-3.

Our findings are consistent with studies undertaken in other countries that have examined transmission in the school setting; when actively sought, transmission to bubble contacts was uncommon, with 1-2% co-primary or secondary infections identified where larger numbers have been sampled (16, 17). It is also consistent with the ~1.5% asymptomatic infection rate reported in a recent cluster-randomised trial of daily lateral flow-testing in bubble contacts (18). The infrequency of transmission to other pupils contrasts with transmission frequency of other respiratory infections in schools, including group A streptococcus and influenza (11,12); this may reflect the multifold interventions in place during the pandemic period, or it may reflect the heterogeneity of infection in COVID19 where most transmission is caused by only a minority of infections (19, 20).

Our study prospectively examined transmission from the same children to contacts in both schools and households; the secondary attack rate in households was higher than expected, and was in stark contrast to that seen in schools. Our findings are consistent with a recent study that reported a secondary attack rate of 25% in households even when the index case is a child (21), and a recent meta-analysis (22). While children may be less likely than adults to become secondary cases, the risk of generating secondary cases is no different whether the index is a child or adult (21, 23); this pattern is confirmed in other countries (22). Quarantine for household contacts, in place throughout our study, may increase exposure of household members to index cases unless mitigated by protective measures, noting household size has been associated with urban caseloads (24). It was notable that in all households with no onward transmission to naïve contacts, householders had ensured that the affected child was isolated from others, without sharing a bedroom, whilst still affording care and supervision.

For ethical reasons, we used GCF to screen for prior SARS-CoV2 exposure, which may under-estimate exposure compared with serum (14). Prevalence of seropositivity among school pupils reported by larger scale testing is similar to levels observed in pupils in our study (25). Due to timing of our study, just six of the adult contacts had been vaccinated. Though vaccination was reported to impact household SAR (26), a recent study suggests a lesser impact with more transmissible variants (27).

Our study adopted a forensic approach to contact tracing, to not miss infections that were cleared early, or those arising late due to ongoing transmission in the class group. We took combined nasal and pharyngeal swabs to increase opportunity for virus detection and used human RNAseP as a control to ensure that negative results could be trusted. Furthermore, almost all swabs were taken by the study team; a small number of contacts were permitted to take swabs themselves if supervised. Genome sequencing identified transmission events that were genuine while also refuting others, highlighting a risk of over- or under-estimating transmission rates when relying on PCR results alone.

The study was designed to investigate bubble sizes of ~10-15, but interpretation of 'bubble' changed over time, and by autumn 2020 bubble sizes routinely included 30-200 primary- and secondary-aged pupils respectively (28). The study relied upon identification of index cases who had been attending school; as such, index cases in this study were almost all symptomatic, with the expectation that asymptomatic cases would be identified among contacts as a comparison group.

There are three key limitations to our study. Firstly, the study was conducted at a time of heightened and constantly changing interventions, in particular social distancing in schools and reduced class sizes. Transmission in schools may alter when interventions relax, as indicated by more recent epidemiological reports (29). Secondly, participation rates in contacts were very low, compared with participation rates of >40% in a previous contact tracing study (12). Deterrents to participation

reported anecdotally were the legal requirement to notify newly-identified infections; quarantine impact on participants; study team making home visits; low risk in children; and inclusion of older pupils. Participation by school contacts was consistently higher than bubble contacts, underlining a resistance to home visits. Recruiting bubble contacts sent home to isolate was challenging, as schools use an array of methods to contact parents. The greatest barrier to participation was the recognition that newly-identified infections would result in quarantine for entire households or classes, such that participation was actively discouraged by some groups, in contrast to predicted responses at study inception. Finally, although our study benefitted from the objective starting point of positive index cases who attend school, there is a risk of bias in all studies that rely on voluntary participation, in terms of individual schools and participants. Representation from a larger number of participants would however require expansive recruitment.

Future research of this kind may provide more meaningful data if the results are unlinked to identifiable data, or any form of notification or requirement to isolate, i.e. without real-time reporting. With reduced interventions and advent of new variants, it may be prudent to evaluate schools-based transmission in such a silent study.

Funding

UKRI/DHSC (Grant COV0322); NIHR

Acknowledgements The study team acknowledge the support of the school leaders, support staff, and the participants who took part in the study as well as the NIHR Clinical Research Networks and Schools Network for supporting the schools in this study.

The input of teaching and parent advisory groups is also acknowledged, as is the practical support of key staff from Imperial College Healthcare NHS Trust, Imperial College London, and Public Health England, and the TraCK study collaborators. We are also grateful to Shamez Ladhani, Samreen Ijaz, John Poh, Justin Shute and members of the Oral Fluid team at PHE Colindale.

Author contributions. Conceptualisation SS and RC; Study site supervision RC; Laboratory supervision GPT, WB, PF, SS; Study methodology SS, RC, GPT, WB, TL, MM; Data collection LR, JZ, AR, PW, CR, MC, MS, LM, AC, GZ, KB, HL, SR, CB, RJ, NA; Data visualisation JZ, LR; Data validation LR, GPT, JZ; Data analysis JZ, JE; Funding acquisition SS, WB, GPT, RC, TL, MM; Writing, original draft SS; Review and critical editing RC, GPT, WB. Final draft: all authors.

Declaration of competing interests The authors declare no competing interests

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Tables

Table 1. Transmission to Bubble classroom contacts

School	Bubble size (incl. cases)	Case number Bubble exposed to	Bubble participant number	Number of bubble contacts testing PCR positive[†]				Bubble contact crevicular fluid anti-NP total IgG	
				Week 1	Week 2	Week 3	Week 4	Positive on first sample	Positive on last sample
A	29	1	3	0/2	0/3	0/3	0/3	1/3	1/3
B	26	1	7	0/4	0/4	0/6	0/7	2/7	1/7
D	41	1	1	0/1	0/1	ND	0/1	0/1	0/1
E[‡]	39	1	2	0/2	0/2	0/2	ND	0/2	0/2
F[§]	48	2	5	0/5	0/5	0/5	ND	0/5	0/5
G[¶]	16	4	2	0/2	0/2	0/2	0/2	0/2	0/2
K[†]	150	11	6	0/6	0/6	0/6	ND	1/6	1/6
M	30	1	2	0/1	0/2	0/2	ND	0/2	0/2
TOTAL			28	0/23	0/25	0/26	0/13	4/28	3/28

[‡]swabbing delayed until 7d after case confirmed.
[§]Includes 2 different bubbles exposed to one case each. One non-participant bubble contact tested positive in community test (included in household study).
[¶]Bubble exposed to 2 adult and 2 child cases
[†] Includes 2 different bubbles exposed to 4 cases and 7 cases
ND, not done

488 **Table 2. Transmission to non-bubble (control) class contacts**
489

School	Non-bubble class size	No. of cases at start of study in school	No. of non-bubble participants	No. of non-bubble contacts testing PCR positive/no. swabbed				Non-bubble contact crevicular fluid anti-NP total IgG	
				Week 1	Week 2	Week 3	Week 4	Positive on first sample	Positive on last sample
A	30	1	5	0/1	0/5	0/5	0/5	3/5	1/5
B	22	1	12	0/12	0/12	0/12	0/12	1/12	1/12
D	27	1	2	0/2	0/1	0/2	0/2	0/2	1/2
E[§]	30	1	10	0/10	3/8	1/5	1/1	2/10	1/8
F	11	2	2	0/1	0/1	0/2	ND	0/2	0/1
G[‡]	24	4	7	ND	0/7	0/7	0/7	1/7	2/7
			3	ND	0/3	0/3	0/3	0/3	0/3
K	306	26	16	0/14	0/16	0/16	ND	3/16	4/16
M	30	1	8	0/6	0/7	0/8	ND	3/8	3/7
TOTAL			65	0/46	3/60	1/60	1/30	13/65	13/61

490 [§]Swabbing of school contacts started one week after initial case
491 [‡]4 cases in school included 2 children and 2 adults. Contacts include 7 children and 3 adults
492 ND, not done
493

Table 3. Transmission events in participating household contacts in each setting

Site	Household size[§]	No. of child cases at start of swabbing[¶]	No. of adult cases at start of swabbing	No. of naïve household contacts	Number of naïve household contacts testing positive				Naïve household contact crevicular fluid anti-NP total IgG	
					Week 1	Week 2	Week 3	Week 4	Positive on first sample	Positive on last sample
A	2	1	0	1	0/1	0/1	0/1	0/1	0/1	0/1
B	3	1	1	1	0/1	0/1	0/1	0/1	0/1	0/1
C[¥]	6	1	0	5	0/5	0/5	0/4	ND	0/5	0/5
D[†▽]	4	1	0	3	2/3	1/3	1/3	0/3	0/3	1/3
E.1[†]	4	1	0	3	0/3	0/3	0/3	ND	0/3	0/3
E.2[‡]	4	1	0	3	1/3	ND	ND	ND	0/3	ND
E.3[†]	7	1	0	6	0/6	ND	ND	ND	0/6	ND
F	4	2	0	2	1/2	2/2	ND	ND	0/2	0/2
G.1[†]	4	1	0	3	0/3	1/3	1/3	0/3	0/3	2/3
G.2[†]	3	1	0	2	1/2	1/2	1/1	0/1	1/2	2/2
H	3	1	2	0	0/0	0/0	ND	ND	0/0	0/0
I	3	1	2	0	0/0	0/0	0/0	ND	0/0	0/0
J	4	2	2	0	0/0	0/0	0/0	ND	0/0	0/0
K.1[†▽]	5	1	0	4	1/4	2/4	0/3	0/2	0/4	1/4
K.2	4	2	2	0	0/0	0/0	0/0	0/0	0/0	0/0
M	3	1	0	2	0/2	0/2	0/2	ND	0/2	0/2
TOTAL				35	6/35	7/26	3/21	0/11	**1/35**	**6/26**

[§] includes index child case: each household had 100% participation rate at time of consent

[¶]includes index child case plus any other child already identified as infected. ND, not done due to intervening holiday or withdrawal from study. Naïve household contacts include 1 child[†]; 2 children[‡]; 3 children[¥]. Household contacts with different genomic sequences to index case are indicated[▽]. Proportions in **bold** are different (p=0.035).

Figure 1. Pictograms of individual contacts in each week of sampling. A. Bubble contacts (n=28). B. School contacts (n=62 pupils, 3 staff) and C. Household cases and contacts. (n=63). For panel C, the 26 participants reported to the study team as having tested positive prior to research swabbing are indicated by* (child index cases, adult and child household contacts). Colour of icons indicates research swab test result in each week of study: Blue icons,negative swab result; pink icons, SARS-CoV2 detected; grey icons, subject not swabbed in that week or not recruited yet. Two of three pupils identified incidentally are included in both panels B and C (i.e. school and household contact pictograms); although pupils were swabbed weekly, the associated households were recruited only after week 2. Within each panel, the figure position is consistent in each week and represents individual participants so can be compared between weeks 1-4. Individual settings are separated by gaps between groups of figures. For presentation purposes, the ordering of settings between panels A, B and C is not the same. Longitudinal sampling was limited to three weeks rather than four weeks for part of the study hence some subjects were not swabbed in week 4.

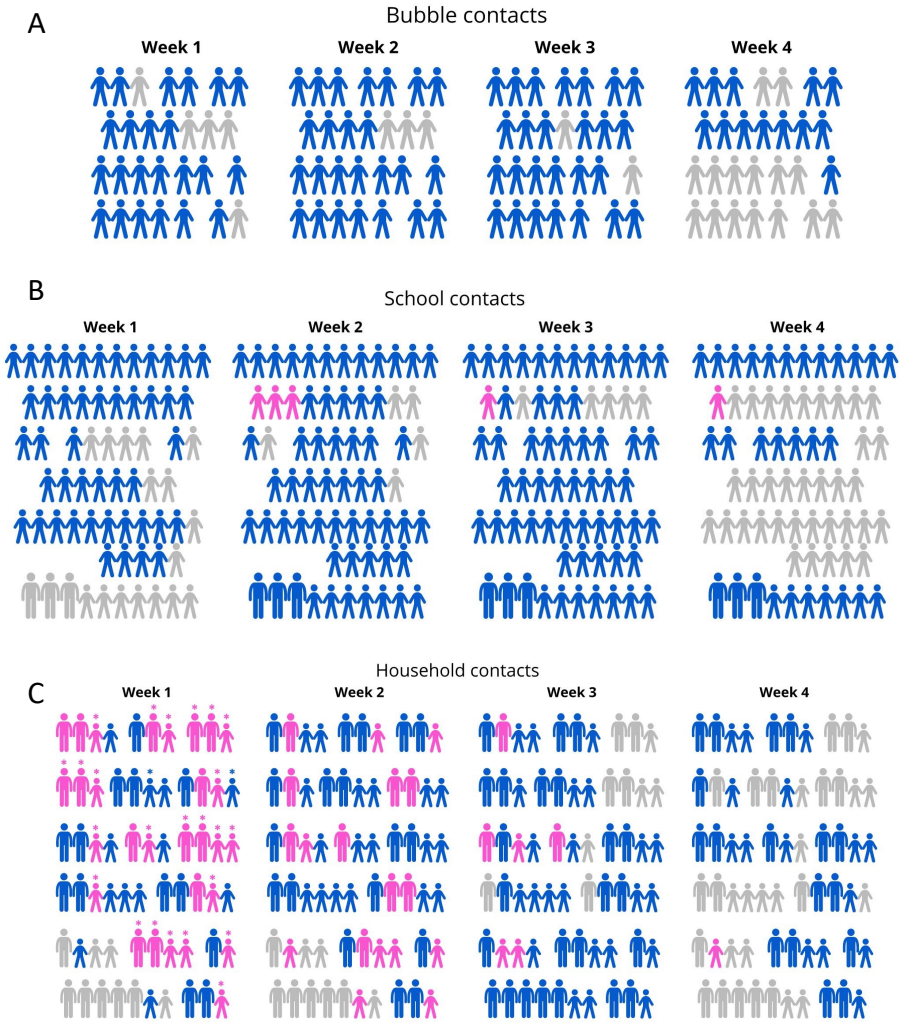

Figure 2.

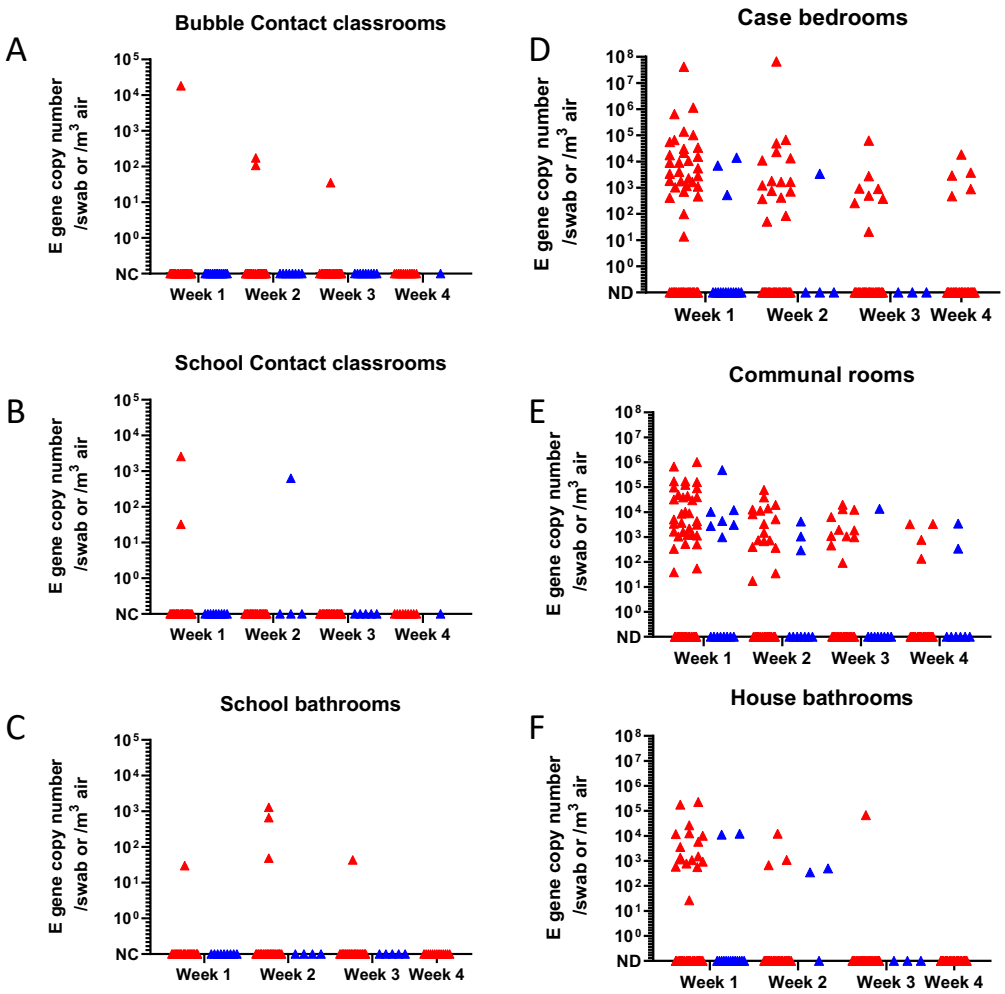

Figure 2 Environmental contamination with SARS-CoV-2 in schools and households. A-C, schools; D-F, households. Samples obtained at start of sampling and thereafter weekly are shown. Red indicates surface samples; blue indicates air samples. Surface and air samples were obtained from the same items and locations weekly in each school and households. Data shown as absolute E gene copy number and represent samples from 8 schools (1 SEND; 2 secondary; 5 primary) and 16 households; note y axis range differs between schools and households A. Bubble contact classroom . B. School contact classroom C. School bathroom used by bubble. D. Child's bedroom. E. Communal room F. Bathroom used by child.

Supplementary Appendix for Transmission of SARS-CoV-2 by children to contacts in schools and households: a prospective cohort and environmental sampling study in London

Supplementary Table 1 Items swabbed in schools and households and university

Household Surface samples		School Surface samples		University building –surface samples	
Case Bedroom	Bed frame	**Classrooms (BC or SC)**	Chair	**Offices**	Chair
	Chair		Desk		Computer
	Computer		Door handle		Desk
	Desk		Hand sanitiser		Door handle
	Door handle		Indoor toys		Food packaging
	Electronic game		Light switch		Light switch
	Laptop		Locker		Mug
	Light switch		Outdoor toys		Printer
	Mobile phone		Reading books		Clothing
	Musical instrument		Soap dispenser		Stationery
	Pillow		Stationery		Personal equipment
	Plastic toys		Student diary		Surgical mask
	School bag		Taps		Telephone
	Soft toys		Window handle		
	Toy shelf		Work folder		
	Wardrobe handle		Work tray		
Bathroom	Door handle	**Washrooms**	Door handle	**Laboratory**	Desk
	Light switch		Soap dispenser		Door handle
	Taps		Taps		Laboratory equipment
	Toilet flush		Toilet flush		Refrigerator handle
	Toilet seat		Toilet seat		Soap dispenser
	Toothbrush and paste				Taps

Communal room	Card game			**Kitchen**	Countertop
	Chair				Cupboard handle
	Door handle				Kettle
	Electronic tablet				Refrigerator handle
	Laptop				Taps
	Light switch				Water machine
	Mobile phone			**Washroom**	Door handle
	Musical instrument				Soap dispenser
	Pet cage				Taps
	Pet fur/feathers[Ψ]				Toilet flush
	Plastic bottle				Toilet seat
	Refrigerator handle			**Lobby & Lifts**	Card reader
	Sofa				Desk
	Soft toys				Door handle
	Stationery				Entry keypad
	Table				Lift buttons
	Taps				Stair handrail
	TV buttons				
	TV remote				
	Wall mirror				
	Water jug				

[Ψ]Included 3x cat fur, 2x dog fur, 1x bird plumage. Abbreviations, BC, Bubble contact. SC, non-bubble school contact

Supplementary Table 2 Environmental sampling results from university

		Surface	Air
Office A[¶]	Sampling 1[‡]	3/10	1/1
	Sampling 2	0/10	0/1
	Total	3/20	1/2
Office B[§]	Sampling 1	0/10	0/1
	Sampling 2	0/10	0/1
	Total	0/20	0/2
Shared offices	Sampling 1	0/10	0/1
	Sampling 2	0/10	0/1
	Total	0/20	0/2
Laboratory	Sampling 1	0/5	0/1
	Sampling 2	0/5	0/1
	Total	0/10	0/2
Kitchen	Sampling 1	0/5	0/1
	Sampling 2	0/5	0/1
	Total	0/10	0/2
Toilets	Sampling 1	0/10	0/2
	Sampling 2	0/10	0/2
	Total	0/20	0/4
Lobby & Lifts	Sampling 1	0/8	0/1
	Sampling 2	0/8	0/1
	Total	0/16	0/2

Second sampling was undertaken 14-15 days after first sampling except in offices A and B

[‡] Values for surface samples were: 7589.1; 31199.7; and 4493.4 E gene copies/swab. Air sample was 3104 E gene copies/cubic metre.

[¶]Second sampling was 12d after first; [§]Second sampling was 3d after first

Supplementary Figures

Supplementary Figure S1 Phylogenetic relation between sequenced SARS-CoV2 isolates from participants with positive swabs

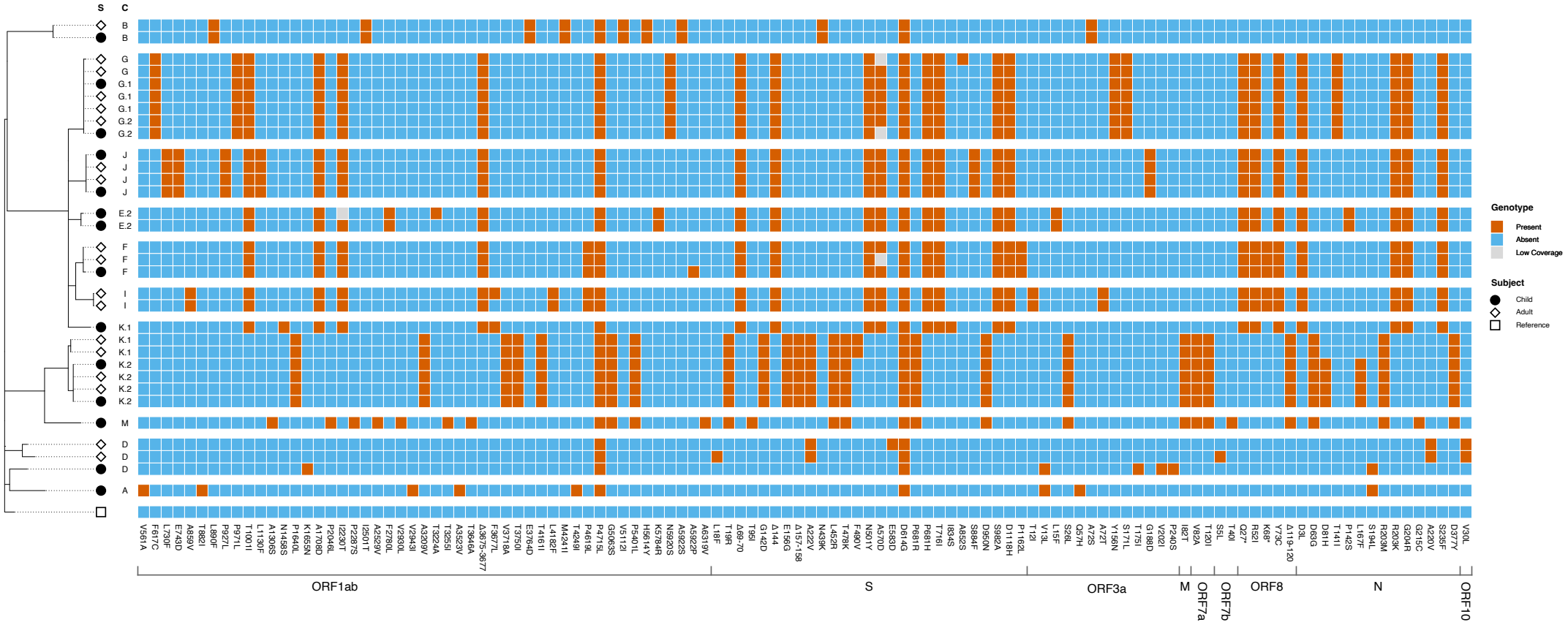

Figure S1. Phylogenetic tree and ORF mutation profile generated through whole genome sequencing of positive SARS-CoV-2 samples from TraCK study participants. The phylogenetic tree is rooted to reference sequence Wuhan-Hu-1 (GenBank accession number NC_045512.2). Samples are grouped by household cluster where possible, always considering phylogenetic tree constraints. S = Subject (Child/Adult/Reference), C = Cluster (setting or household).

Supplementary Figure S2 Surface contamination with SARS-CoV2 in households by category over sampling period.

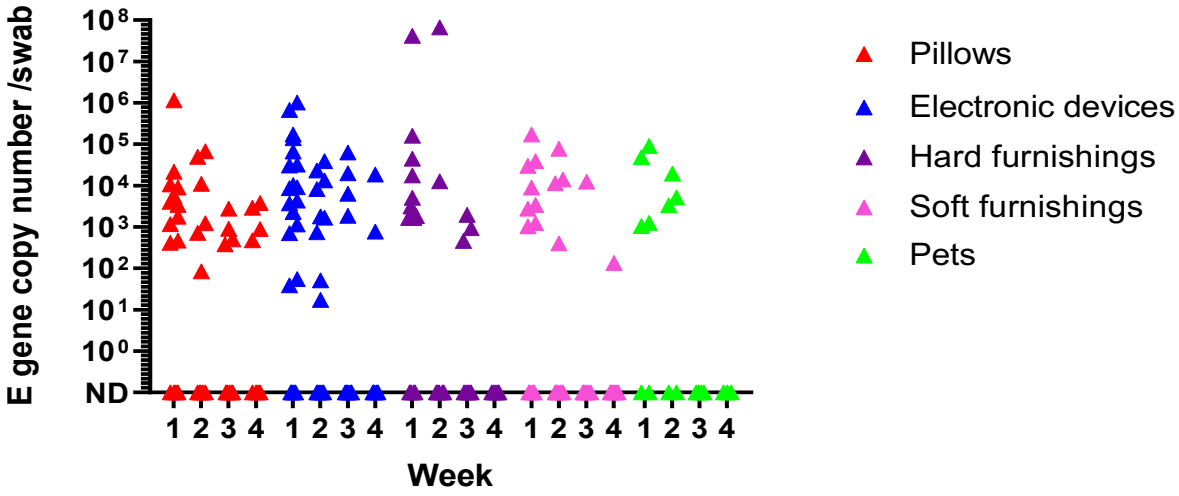

Figure S2. Environmental samples from 16 households by item category as listed in legend. E gene copy number per swab is shown for each item at each weekly time point. All items swabbed within a household were consistently sampled again on each sampling occasion within a given household; some households were swabbed for less than 4 weeks. Pet sampling included 3x cat fur, 2x dog fur, 1x bird plumage but no mucosal sampling.

Supplementary Figure S3 Comparison of human target detection in household and school environmental samples

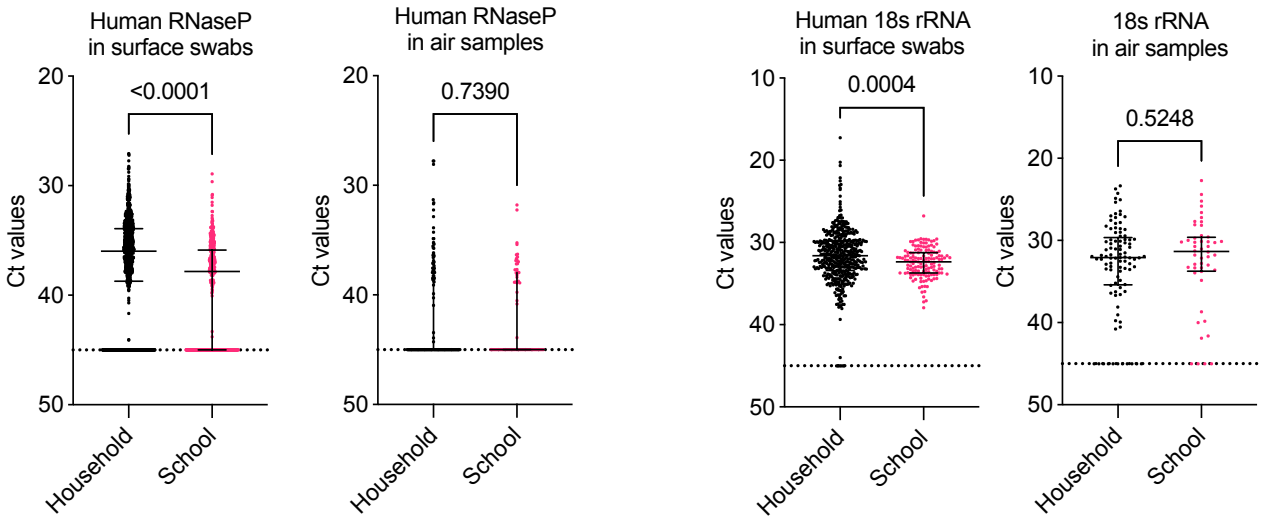

Figure S3. Human RNaseP and 18s rRNA detected in surface swabs and air samples collected from schools and households. Surface and air samples were obtained from the same items and locations weekly in each school and households. Data are shown as median and IQR Ct values determined by real-time PCR. Results between household and schools samples were compared using Mann-Whitney U test (GraphPad Prism) were shown with corresponding p values as indicated.

Supplementary Methods

Context for Case and Bubble definitions and quarantine periods

During the study period, children in England were tested for SARS-CoV-2 by PCR if exhibiting any of the recognised symptoms of COVID-19 through community or postal testing programmes. From September 1st 2020 – July 19th 2021, schools and nurseries were required by the UK government to undertake contact tracing for suspected or confirmed cases of COVID-19 in pupils or staff. Children with confirmed SARS-CoV-2 infection were excluded from onset of symptoms (or a positive test if no symptoms). The duration of exclusion was initially 14 days (1 Sept 2020 – 14 Dec 2020) later changing to 10 days (14 Dec – 19 July 2021). The same duration of quarantine applied to household contacts of cases regardless of vaccination status. Contacts identified by schools were excluded for the same duration. In early years and primary school settings the whole class were considered close contacts (the so-called "bubble"). In secondary school settings risk assessment identified individual close classroom contacts (face to face contact; contact within 1m for >1 minute; within 2m for >15 minutes).

Prevailing interventions in schools and school closures.

Schools in England re-opened in the first week of September 2020 to all children aged 5-18, having adopted a suite of preventive measures including social distancing, hand hygiene, and secondary school-aged pupils were required to wear masks when not in class; any positive cases arising in schools resulted in bubble contacts quarantining for 14 days. Schools closed in mid December 2020 for the Christmas holidays. Between January 4th and March 8th 2021 schools in England partially re-opened for vulnerable children, children of keyworkers, and secondary school-aged pupils undertaking exams in years 11 and 13 only. From March 9th 2021 schools re-opened to all pupils and, in addition to the aforementioned measures, secondary school-aged pupils were required to undertake lateral flow antigen testing for SARS-CoV-2 twice weekly and wear masks inside and outside the classroom.

Contact definition.

Bubble contacts (BC) were children identified by schools who were required to isolate at home. For nurseries and primary schools, BC were in the same 'bubble' or class as the index case; for secondary schools, BC had been individually identified by the school as meeting the contact definitions above.
Non-bubble school contacts (SC) were children from a different 'control' class in the same school. SC were from a class that was adjacent in terms of age-group or geographical proximity in the school. They had not been identified by school as contacts required to isolate, but were drawn from the same wider community and, despite best efforts to keep bubbles separate, may have been exposed to similar common areas in the school as the index case the BC. Household contacts (HC) were adults and children of any age normally resident with the Case, and required to isolate.

Sample size.

The study was pragmatic in that it enrolled as many bubble contacts as possible within the school year. A prevalence of 25% infection was previously detected in in classroom contacts exposed to scarlet fever (1). A sample size of 40 bubble contacts was sought to detect a difference between the Null hypothesis proportion, $\pi_0$of 0.03 and the Alternative proportion, π_1, of 0.25 with 98.4% power using an exact binomial test with a nominal 5% two-sided significance level; for a sample size of 28, power was 94.49%.

Contact sampling

Combined nose and throat samples were obtained by the research team from each participating contact (BC, SC, or HC) as soon as possible (<48 hours) after case identification, and thereafter weekly for up to 28 days. Flocked nylon swabs (Sterilab Services, Harrogate, UK) were rubbed on the posterior fauces and then rotated gently in the nostrils no deeper than the length of the flocked end of the swab, then placed into universal transport medium. BC and HHC were sampled at home by the study team, while SC were sampled at school by the same study team. Swabs were delivered to the laboratory the same day and immediately refrigerated until processed the following working day.

Environmental sampling.

For households, surface and air samples were obtained in each of three rooms (child's bedroom, communal room, bathroom) weekly. For schools, surface and air samples were obtained from the bubble classroom, school contact classroom, and washroom weekly. Details of environmental samples obtained are listed in supplementary table 1.

For environmental surface sampling, swabs moistened in viral transport medium were used to swab 25 cm^2 of four or five surfaces in each of three rooms (child's bedroom, communal room, bathroom), identified as frequently touched or handled by the case, with attention on personal items (total 14 swabs). Where household pets were available, surface samples (fur or feathers) were obtained from these at the same time as other household items; mucosal samples were not obtained.

Air sampling was undertaken in the same three rooms for periods of 10 minutes (300 litres/minute, Coriolis micro, Bertin Instruments, France), with the Case present in the communal room during sampling. Environmental sampling in the home started at time of household recruitment and surfaces were re-swabbed weekly for up to 28 days at the time of household sampling.

For schools, surface swabs were taken from four or five surfaces in three locations: Bubble classroom (n=5); School contact classroom (n=5); Washroom (n=4). Schools were asked to delay cleaning of bubble classrooms until after the week 1 swabs were taken but this was not always possible. Surfaces were re-swabbed weekly for up to 28 days. Air sampling was undertaken in the same three locations, repeated weekly. Where children were present in school, sampling was undertaken immediately after children had left the class.

For the university building, surface swabs were obtained on two occasions from two academic offices; a research laboratory; washroom; kitchen area; elevator and communal lobby area.
Environmental samples were coded then tested by a research laboratory for SARS-CoV-2 RNA content using a quantitative RT-PCR detecting SARS-CoV-2 E and Orf1ab genes (2) using human RNAseP and 18s rRNA as controls for sample quality and as an indicator of human contact. Samples with high SARS CoV2 viral load (Ct value <30) were inoculated into Vero cells for culture of infectious virus as previously reported (2).

Whole genome sequencing, lineage assignments and phylogenetic trees

RT-qPCR was performed using an in-house protocol (3). Samples with a positive RT-qPCR result were submitted for Whole Genome Sequencing to assign lineages and generate phylogenetic trees. Samples with the highest viral loads were chosen. Automated RNA extraction was performed using a CyBio FeliX (Analytik Jena) and innuPREP Virus TS RNA Kit 2.0 (Analytik Jena) according to the manufacturer's instructions, with a sample volume of 200 µl, without carrier RNA and with an elution volume of 50 µl. cDNA synthesis was then performed using the LunaScript RT SuperMix Kit (NEB) according to the manufacturer's instructions with a total reaction volume of 20 µl and extracted sample volume of 5 µl. Libraries were generated using the EasySeq™ RT-PCR SARS CoV-2 (novel coronavirus) Whole Genome Sequencing kit v1 or v2 (Nimagen) according to the manufacturer's instructions. Samples were then pooled and purified with AMPure XP (Beckman Coulter) magnetic beads. Suitable quality of libraries was confirmed using a Tapestation (Agilent) and concentrations were measured using the Qubit 1x dsDNA High Sensitivity Assay Kit (Thermofisher Scientific) and Qubit 4 Fluorometer (ThermoFisher Scientific). Pooled libraries were then diluted down to 55 pM. The final pool was then run on an iSeq 100 (Illumina) with a total of 322 cycles (151 bp paired reads and 10 bp indices). Generated fastq files were processed using the EasySeq variant pipeline (v0.6.0)(4) which is a Nextflow (5) pipeline that uses fastp (6), BWA MEM (7), SAMtools (8), BCFtools (8), LoFreq (9), mosdepth (10), BEDtools (11), SnpEff (12) and MultiQC (13) to QC, trim and assemble the reads (using reference sequence NC_045512.2) and then generate a consensus sequence and variant report before assigning a PANGO lineage (14) using pangolin (v3.1.16, lineages version 2021-10-18) (15). Sequences were aligned using Clustal Omega (16) and the alignment was then used to generate a phylogenetic tree using IQ-TREE (v2.1.3) (17). The phylogenetic tree and heatmap were generated using R (18) and the ggtree package (19).

Gingival Crevicular Fluid (GCF). GCF was collected from each participant at each swabbing time point (Oracol swabs, Malvern Medical, Worcester, UK). Foam swabs were rubbed on the gums for one minute at each sampling time point stored at 4° C until elution in transport medium (phosphate-buffered saline (PBS), supplemented with 10% fetal calf serum, 0.2% Amphotericin B, and 0.5% gentamicin) and then stored at -20°C until analysis by the reference laboratory (20).

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