Research paper

Antibody persistence and neutralising activity in primary school students and staff: Prospective active surveillance, June to December 2020, England

Georgina Ireland[a,*], Anna Jeffery-Smith[a], Maria Zambon[a], Katja Hoschler[a], Ross Harris[a], John Poh[a], Frances Baawuah[a], Joanne Beckmann[c], Ifeanyichukwu O Okike[a,d], Shazaad Ahmad[e], Joanna Garstang[f], Andrew J Brent[g,h], Bernadette Brent[g], Felicity Aiano[a], Zahin Amin-Chowdhury[a], Louise Letley[a], Samuel E I Jones[a], Meaghan Kall[a], Monika Patel[a], Robin Gopal[a], Ray Borrow[i], Ezra Linley[i], Gayatri Amirthalingam[a], Kevin E Brown[a], Mary E Ramsay[a], Shamez N Ladhani[a,b]

[a] Immunisation and Countermeasures Division, National Infection Service, Public Health England, 61 Colindale Avenue, London NW9 5EQ, United Kingdom
[b] Paediatric Infectious Diseases Research Group, St. George's University of London, London SW17 0RE, United Kingdom
[c] East London NHS Foundation Trust, 9 Allie Street, London E1 8DE, United Kingdom
[d] University Hospitals of Derby and Burton NHS Foundation Trust, 201 London Road, Derby DE1 2TZ, United Kingdom
[e] Manchester University NHS Foundation Trust, Oxford Road, Manchester M13 9WL, United Kingdom
[f] Birmingham Community Healthcare NHS Trust, Holt Street, Aston, B7 4BN, United Kingdom
[g] Nuffield Department of Medicine, Oxford University Hospitals NHS Foundation Trust, Old Road, Oxford OX3 7HE, United Kingdom
[h] Wellington Square, University of Oxford, Oxford OX1 2JD, United Kingdom
[i] Public Health England, Manchester Royal Infirmary, Manchester, United Kingdom

ARTICLE INFO

ABSTRACT

Background: Prospective, longitudinal SARS-CoV-2 sero-surveillance in schools across England was initiated after the first national lockdown, allowing comparison of child and adult antibody responses over time.

Methods: Prospective active serological surveillance in 46 primary schools in England tested for SARS-CoV-2 antibodies during June, July and December 2020. Samples were tested for nucleocapsid (N) and receptor binding domain (RBD) antibodies, to estimate antibody persistence at least 6 months after infection, and for the correlation of N, RBD and live virus neutralising activity.

Findings: In June 2020, 1,344 staff and 835 students were tested. Overall, 11.5% (95%CI: 9.4–13.9) and 11.3% (95%CI: 9.2–13.6; $p = 0.88$) of students had nucleoprotein and RBD antibodies, compared to 15.6% (95%CI: 13.7–17.6) and 15.3% (95%CI: 13.4–17.3; $p = 0.83$) of staff. Live virus neutralising activity was detected in 79.8% ($n = 71/89$) of nucleocapsid and 85.5% (71/83) of RBD antibody positive children. RBD antibodies correlated more strongly with neutralising antibodies (rs=0.7527; $p<0.0001$) than nucleocapsid antibodies (rs=0.3698; $p<0.0001$). A median of 24.4 weeks later, 58.2% (107/184) participants had nucleocapsid antibody seroreversion, compared to 20.9% (33/158) for RBD ($p<0.001$). Similar seroreversion rates were observed between staff and students for nucleocapsid ($p = 0.26$) and RBD-antibodies ($p = 0.43$). Nucleocapsid and RBD antibody quantitative results were significantly lower in staff compared to students ($p = 0.028$ and <0.0001 respectively) at baseline, but not at 24 weeks ($p = 0.16$ and $p = 0.37$, respectively).

Interpretation: The immune response in children following SARS-CoV-2 infection was robust and sustained (>6 months) but further work is required to understand the extent to which this protects against reinfection.

Funding: Department for Health and Social Care.

1. Introduction

SARS-CoV-2 first emerged in December 2020 and spread rapidly across the globe, causing more than 100 million cases within 12 months, with more than 2 million deaths, mainly amongst the elderly

* Corresponding author.
E-mail address: geoergina.ireland@phe.gov.uk (G. Ireland).

Research in context

Evidence before this study

A PubMed search found children develop robust humoral and cellular immune responses after symptomatic and asymptomatic SARS-CoV-2 infection. Younger children have higher antibody titres but lower neutralizing activity than older children or young adults. There are however limited data on the longevity of antibody and cellular immune responses following acute infection in children.

Added value of this study

Through the serial collection of blood samples in primary school students and staff over a 6-month period, we found that SARS-CoV-2 antibody seropositivity rates were similar in students and staff, with higher antibody results at baseline but similar results 6 months later. Nearly all students and staff retained SARS-CoV-2 antibodies after 6 months.

Implications of all the available evidence

Young children develop and maintain robust and sustained immune responses that is at least similar to adults after acute infection. Further studies are needed to assess protection against emerging variants of concern.

[1,2] In order to limit the rapid spread of the virus, many countries implemented national lockdown measures including school closures which, in England, began on 20 March 2020. Children, however, have a lower risk of disease, hospitalisation or death due to COVID-19 compared to adults [3]. Indeed, most children exposed to SARS-CoV-2 remain asymptomatic or develop mild, transient infection with non-specific symptoms, which may often not be attributed to the virus, raising concerns that they may unknowingly transmit the virus to others [4].

Following partial reopening of UK schools after national lockdown in June 2020, there were concerns that the large number of children gathering in close confinement with limited social distancing would provide a hub for infection, leading to widespread transmission, not only amongst the children themselves, but also to school staff, household members and, potentially, the wider community [5,6]. In order to investigate SARS-CoV-2 infection and transmission in educational settings, Public Health England (PHE) initiated prospective active surveillance in 131 primary schools (5−11 years) across England. Some schools implemented weekly swabbing for participating staff and students, while others involved swabbing with blood sampling to measure serum SARS-CoV-2 antibodies [7].

Antibody testing provides a robust measure of prior exposure to SARS-CoV-2 in individuals with symptomatic, asymptomatic or mild, transient infection, making it a useful surveillance tool. Antibodies, and neutralising antibodies in particular [8], are also important markers of immunity against re-infection. Recent longitudinal adult studies, mainly in healthcare workers and adults, have shown that SARS-CoV-2 antibodies following SARS-CoV-2 infection lasts for at least 6−8 months [9], and is associated with up 90% protection against re-infection [10]. Antibody persistence after infection is, therefore, an important component in assessing duration of protection. This is particularly the case in children who can have multiple re-infections with endemic coronaviruses, at least in part due to antibody waning [11,12] Evidence to date has demonstrated antibody persistence in children up to 62 days after SARS-CoV-2 infection, but data on long-term antibody persistence in children is limited [13]. The COVID-19 **S**urveillance in school **KID**s (sKIDs) study, collected 3 samples between June and December 2020, and, to date, the analysis

of these data has focused on transmission within schools. However, the repeat samples also provide a unique opportunity to investigate the persistence of SARS-CoV-2 antibodies against nucleocapsid (N) and the receptor binding domain (RBD) of the spike protein at least 6 months after infection in primary school students, and compare them to staff [7]. We additionally investigate N and RBD-antibody seroprevalence differences in the first sampling round, and the correlation between N, RBD and live neutralising antibodies.

2. Methods

2.1. Sampling

The protocol for sKIDs surveillance is available online [7]. Briefly, 131 primary schools were recruited and included 46 schools involved in swabbing with blood sampling at three timepoints: following partial reopening of schools on 01 June 2020 (1−19 June 2020) and end of the summer term (3−23 July 2020), which were used as baseline seroprevalence and, following full reopening of all schools on 1 September 2020, at the end of the autumn term (23 November to 18 December 2020). Informed consent was provided by staff and the parent or guardian of students prior to the first sampling round in participating schools. The protocol was approved by PHE Research Ethics Governance Group (Ref: NR0209, 16 May 2020). The primary aim of this analysis was to estimate antibody persistence at 6 months in children, with secondary analysis aims of comparing N and RBD antibody seroprevalence and the correlation of RBD, N and neutralising antibodies at the start of the study.

2.2. Laboratory testing

Blood samples were tested for nucleoprotein antibodies using a chemiluminescent microparticle immunoglobulin G (IgG) immunoassay targeting the nucleocapsid (SARS-CoV-2 IgG, Abbott Commerce Chicago, USA) with a seropositivity cut-off index (COI) threshold of 0.8. The samples were also tested for RBD antibodies using an in-house indirect IgG RBD assay, seropositivity COI threshold of 5 [14]. Commercial RBD subunit was purchased from SinoBiological Inc (Beijing, P.R. China) and expressed in HEK293 cell culture with a C-terminal mouse Fc tag (Arg319-Phe541 (V367F);# YP_009724390.1). Nunc MaxiSorp flat-bottomed, polystyrene 96-well microtitre plates were coated by diluting 20 ng recombinant protein/well in sterile phosphate buffered saline (PBS); pH7.2 $\pm$ 0.05 (-CaCl2, -MgCl2), (GIBCO, Thermo Fischer, Waltham, U.S.A) at 4−8 °C for a minimum of 16 h. Serum was diluted at a final dilution factor of 1 in 100. IgG binding on the plate surface was detected with an anti-Human IgG−horseradish peroxidase antibody conjugate (Sigma Aldrich, St Louis, U.S.A) and detected with 3,3′, 5,5′- Tetramethylbenzidine (Europa Bioproducts Ltd, Ipswich, U.K). Samples were analysed in duplicate and optical density (OD450) data were evaluated by dividing average OD450 values for individual samples by average OD450 of a known calibrator with negative antibody levels (T/N ratio).

Blood samples with sufficient serum from N antibody positive students in June and July 2020 were also tested for neutralising antibodies using a modification of the WHO influenza microneutralisation methodology [15]. Briefly, 200 median tissue culture infectious dose (TCID50) of virus was incubated with serial dilutions of serum from participants, after which a suspension of Vero E6 cells were added. After 22 h, cells were fixed and in-cell SARS-CoV-2 N expression determined by ELISA using Rabbit polyclonal to SARS-CoV N (Sino-Biological; Cat no 40,143 T62). The live virus-neutralising antibody titre was determined as the serum concentration that inhibited 50% of SARS-CoV-2 N expression.

2.3. Statistical analysis

Data were analysed using Stata SE (version 15.1). Student and staff seropositivity for N and RBD antibodies was compared and antibody persistence at 24 weeks was estimated for participants tested in June 2020 and at the end of the autumn term in November/December 2020. Where participants were also tested in July, antibody persistence at 4 weeks was estimated. Whilst seroprevalence comparison in June is a secondary aim, it is presented first in the results, as the analysis of antibody longevity at 4 and 24 weeks, the primary aim, is conducted on a subset of this group.

Seroreversion was defined as testing negative after previously testing positive on the same antibody assay platform. Categorical variables were described as proportions and compared using Chi [2] and Fisher's Exact tests. Seroprevalence and antibody persistence proportions were calculated with 95% binomial confidence intervals. Spearman's rank was used to test for correlation between neutralising antibody response, N-antibody and RBD-antibody results. Correlation between longitudinal samples, taken on average 4 weeks later, was also tested for. Kruskal-Wallis H Test was used to compare neutralising results by age-group in children. Due to the rapid initiation of the study in June, age was not well complete, but following efforts to improve data quality age is known for 96% of students and 69% of staff. As a result of missing data and a focus on child antibody-results, age comparisons are on students only. Previous studies have shown some linear differences in N and spike antibody levels by age in children, so students were split into three equally sized age-groups [16,17].

Quantitative antibody results were log-transformed and compared using matched and unmatched t-tests. Additionally, a linear regression on log-transformed quantitative results was used to test for differences between students and staff, and at baseline and final sample, including an interaction between participant type and testing round. For RBD, this only included participants who were RBD positive at baseline. Samples were clustered by individual participant to account for repeated measurements. The regression coefficient was exponentiated to provide ratio measures.

2.4. Role of funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. SNL and GI had access to the data and had final responsibility to submit for publication.

3. Results

3.1. Seroprevalence in June

At recruitment in June 2020, 2179 samples obtained from sKIDs participants in England had sufficient sample volume to be additionally tested for RBD antibodies (Fig. 1). Staff represented 61.7% (1344/2179); median age was 43 years (interquartile range [IQR], 33−52) and 80.8% (1086/1344) were females. The median age of 835 students (38.3%) was 8 years (IQR: 6−10) and 50.5% (422/835) were females.

Overall, seropositivity for N (14.0%; 305/2179; 95% Confidence Interval [CI]: 12.6−15.5) and RBD (13.7%; 299/2179; 95% CI: 12.3−15.2) antibodies was similar (p = 0.79). This was also true for students and staff: 11.5% (95% CI: 9.4−13.9) and 11.3% (95% CI: 9.2−13.6; p = 0.88) of students were seropositive for N and RBD antibodies, respectively, compared to 15.6% (95% CI: 13.7−17.6) and 15.3% (95% CI: 13.4−17.3; p = 0.83), respectively, amongst staff (Table 1). N and RBD-antibody quantitative results correlated significantly (0.4280, p<0.0001) (Fig. 2).

N and RBD-antibody result agreement was 96.6% (Fig. 1b), with a lower proportion of staff having concordant results compared to students (95.5% vs 98.3%; p<0.001). Of the 74 (3.4%) with discordant results, 34 (45.9%) were N-antibody negative but RBD-antibody positive, 40 (54.1%) were N-antibody positive but RBD negative and there
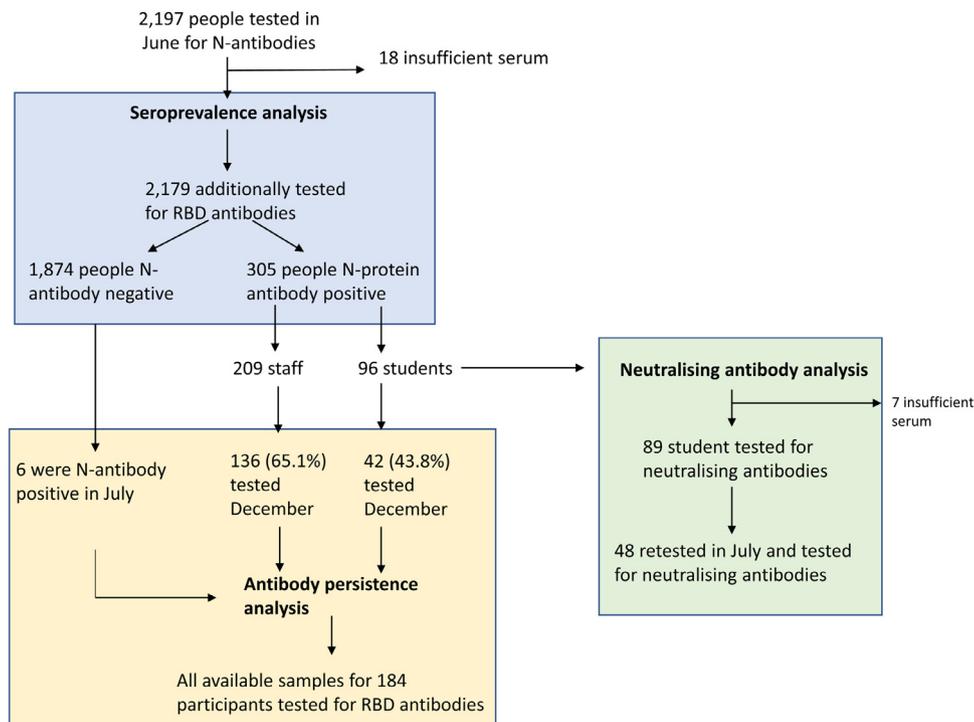

Fig. 1. Participant flow between antibody seroprevalence, antibody persistence and neutralising antibody analysis in sKIDs participants (a) and agreement between N and RBD antibody results in participants tested in June 2020 on both assays (n = 2179) (b).
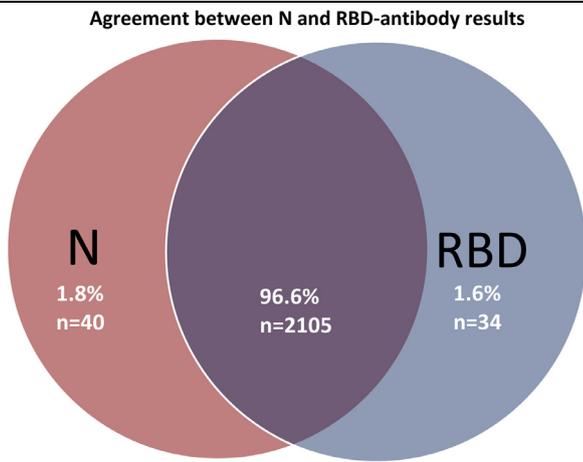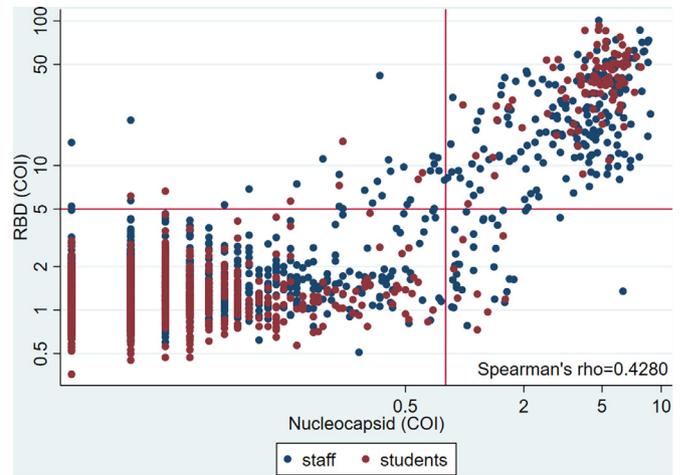
Fig. 1. Continued.

Fig. 2. Log nucleocapsid and RBD antibody quantitative results (COI) for 2179 students (red) and staff (blue) tested as part of the sKIDs study in June 2020 in England. Lines denote the threshold values for reporting a positive result.

was no difference in proportion with discordant results between students and staff (p = 0.52).

Live virus neutralising antibody titres were assessed in 89 N-antibody positive students from the June testing round. In these samples, 79.8% (71/89) had neutralising antibodies and all six children (6.7%) who were RBD antibody negative at baseline had no detectable neutralising antibodies. Of the 83 RBD-antibody positive students at baseline, 85.5% (71/83) had neutralising antibodies. One month later, a paired sample was available and tested for neutralising activity in 48 students. Across all samples (n = 137), both N and RBD quantitative results positively correlated with neutralising titres, with RBD antibodies demonstrating a stronger correlation (N: 0.3698, $p<0.0001$ and RBD: 0.7527, $p<0.0001$). (**Supplementary Fig. 1a and 1b**). There was no difference in neutralising antibody titres by age in students (p = 0.30) (**Supplementary Fig. 2**).

Median neutralising antibody titres in 48 students with paired samples in June and July were similar (35.2 [IQR,26.0−54.1] in June vs. 33.0 [IQR:22.5−49.8] in July; p = 0.23) and correlated strongly between the two-time points (0.8087; $p<0.0001$) (**Supplementary Fig. 1c**).

3.2. Nucleocapsid and RBD-antibody persistence

Of the 311 participants (211 staff, 100 students) who were N-antibody positive in June 2020 or seroconverted by July 2020, 184 (59.2%) underwent subsequent sampling at the end of the autumn term. This cohort included 178 participants who were N-antibody positive in June and 6 who had seroconverted by July (Fig. 1).

Of the 178 who were N-antibody positive in June, 69.7% (124/178) had a further antibody sample in July (median 4.3 weeks, IQR: 3.9−5.1). Of these, none of the students seroreverted for N (0/25) or RBD (0/24) antibodies, whereas, 5.1% (5/99) of staff seroreverted for N and 10.0% (8/80) for RBD antibody (Fig. 2). Only one of the 5 staff participants with N antibody seroreversion remained positive for RBD antibodies.

At the end of the autumn term, 184 (46 students, 138 staff) had a repeat blood sample at a median of 24.4 weeks (IQR: 23.1−25.9) later. Of these, 58.2% (107/184) seroreverted for N-antibodies compared to 20.9% (33/158) for RBD ($p<0.001$) (Fig. 3). There was no difference in N (65.2% vs 55.8%, p = 0.26) and RBD antibody (16.7% vs 22.4% p = 0.43) seroreversion between students and staff. Of those who seroreverted on the N antibody assay, 73.3% (n = 22/30) of students and 59.7% (n = 46/77; p = 0.19) of staff remained positive for RBD antibodies.

In this cohort of 184 N antibody positive participants, 26 (14.1%) had a negative RBD result in June 2020, including 4 (4/46, 8.7%) students and 22 (22/138, 15.9%) staff (p = 0.22). Only one, a staff member (1/22, 4.5%), subsequently seroconverted on the RBD assay by the end of the autumn term.

The N and RBD antibody quantitative results were significantly lower in staff compared to students (p = 0.028 and <0.0001 respectively) at baseline, but not at 24 weeks (p = 0.16 and p = 0.37, respectively) (Fig. 4). Using linear regression, N-antibody results at baseline were 22.8% (95% CI: 6.0−35.0%) lower in staff than students and 42.1% (95% CI: 29.3−52.6) lower against RBD. After 6 months, N-antibody results were 88.2% (95% CI: 83.4−91.7) lower compared to baseline results in students and 78.4% (95% CI: 75.0−81.4) lower amongst staff. The corresponding reductions for RBD antibody results were 69.0% (95% CI: 58.5−76.9) and 50.4% (95% CI: 43.7−56.4), respectively. Only 3.3% (6/184) and 10.8% (17/158) had an increase in N and RBD quantitative results respectively at 24 weeks than baseline.

4. Discussion

SARS-CoV-2 antibody prevalence was similar in primary school staff and students in June 2020, with high concordance between antibody seropositivity against N and the RBD of the spike protein. Antibody kinetics over time, however, differed. Less than half of positive

Table 1
Nucleocapsid and RBD antibody seropositivity at baseline in primary school students and staff during June 2020.

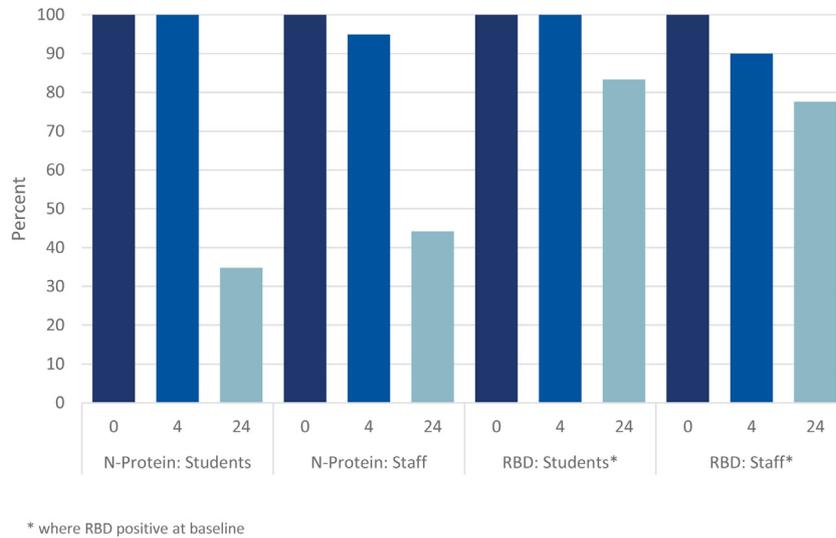|		RBD antibody					
		Total		Students		Staff	
		Negative	Positive	Negative	Positive	Negative	Positive
Nucleocapsid antibody	Negative	1840 (84.4)	34 (1.6)	733 (87.8)	6 (0.7)	1107 (82.4)	28 (2.1)
	Positive	40 (1.8)	265 (12.2)	8 (1.0)	88 (10.5)	32 (2.4)	177 (13.2)

Fig. 3. Nucleocapsid and RBD-antibody* positivity at 0, 4 and 24 weeks in the sKIDs Study in England.
   *where RBD positive at baseline.

participants retained N-antibodies measured using the Abbott assay. RBD-antibodies, on the other hand, correlated more strongly with neutralising antibody titres, and persisted in 78% of students and 83% of staff for more than 6 months after infection. Antibody results at baseline were significantly higher in students than staff but declined over time such that they were similar in the two groups after 24 weeks.

The Abbott N-antibody assay was originally selected for SARS-CoV-2 surveillance in educational settings because it could detect SARS-CoV-2 antibodies within 7−14 days after infection, earlier than assays measuring Spike protein antibodies, thus facilitating early detection of seroconversions after symptomatic or asymptomatic infections between testing rounds [6,18,19] There is, however, increasing evidence that the high seroreversion rates observed with the Abbott assay are not biological but a function of the antibody assay. In our other adult cohort studies, for example, 44% of adults seroreverted for N-antibodies using the Abbott assay by 9 weeks and 58% by 24 weeks [18] but N-antibodies remained positive using other assays such as the Roche N assay [18] Other investigators have also reported similar findings [20] Nucleocapsid is an immunodominant antigen in many enveloped virus infections and, although less well correlated with neutralising, nucleoprotein antibodies to other viruses have been shown to facilitate viral clearance *in vivo*, most likely through T-cell mediated immunity and cytotoxic T cells (CTLs) against nucleoprotein, which can rapidly eliminate infected cells displaying nucleoprotein peptides [21].

Because of the high seroreversion rate for N-antibodies with the Abbott assay, we re-tested all available sera with a validated in-house RBD assay, which we and others have shown to correlate more strongly with neutralising antibodies in adults [14,22,23]. We have now also confirmed this strong correlation in young children. Moreover, other investigators found almost all adults with confirmed COVID-19 had neutralising antibodies up to 6 months later and, while antibody results decreased progressively over time, anti-RBD antibody concentrations and neutralising antibody titres remained strongly correlated at 1, 3 and 6 months after infection [24]. This is reassuring for our cohort as the majority of students and staff will have been infected with SARS-CoV-2 during the first pandemic peak in the UK in March and April 2020, and retained RBD antibodies up to nine months later, at the end of the autumn term in November/ December 2020.

A small proportion of participants in our cohort had detectable N-antibodies but were RBD antibody negative upon initial testing and, importantly, had no virus neutralising activity and remained RBD-antibody negative in subsequent tests. Taken together, these findings suggest that a small proportion had false positive antibody results using the Abbott assay, potentially due to non-specific, cross-reactive antibodies from prior coronavirus infections [18]. The choice of antibody assay in sero-surveillance will become increasingly important as current vaccines only induce spike protein antibodies, while measurement of N-antibodies will help confirm prior SARS-CoV-2 infection irrespective of vaccination status.
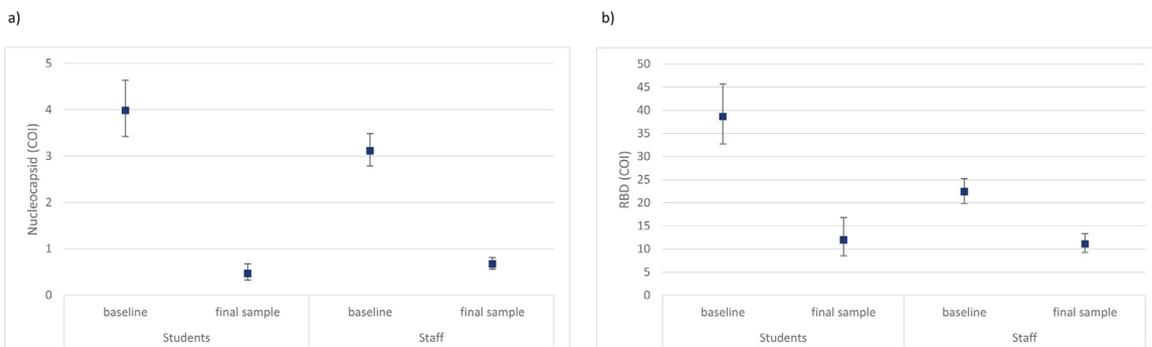

Fig. 4. Geometric mean, with 95% confidence intervals, of (a) nucleocapsid and (b) RBD* antibody quantitative results (COI) at baseline and 24 weeks (final sample) for students and staff participating in the sKIDs study. *where RBD antibody positive at baseline.

Compared to staff, students had significantly higher RBD and N antibody results at baseline, but these differences had resolved after six months, with similar RBD antibody results and seroreversion rates during this period. This is reassuring, given that 76% of student compared to 47% of staff who seroconverted during the surveillance period had asymptomatic infection, indicating that children with asymptomatic SARS-CoV-2 infection are able to mount a robust and durable immune response similar to – if not better than – adults [7].

We assessed the association between antibody results and virus neutralising activity in students only because this has already been extensively investigated in adults. The few studies in children have reported robust immune responses following SARS-CoV-2 infection *albeit* with some important differences when compared with adults [16,17] amongst 1−24 year-olds in a recent New York seroprevalence study, for example, SARS-CoV-2 total IgG and RBD antibody titres were highest in younger children and declined with increasing age, while surrogate neutralising antibody activity and antibody avidity were lowest in younger children and increased with age [17]. Others have also reported lower neutralising antibody titres in children compared to adults [16,17,25]. We have, however, recently reported a higher prevalence and magnitude of cellular responses against the spike protein of SARS-CoV-2 in our cohort of primary school-aged children compared to adults more than six months after primary infection [26].

The early initiation of surveillance in primary schools provided a unique opportunity to monitor seroprevalence, seroconversion and antibody persistence in more than 2000 healthy young children and adults with similar exposure risks to SARS-CoV-2 in 45 schools across England [7]. A limitation of our study was the limited testing for SARS-CoV-2 infection in the community during the first wave of the pandemic; we were, therefore, unable to confirm acute SARS-CoV-2 infection in symptomatic participants prior to recruitment. Additionally, most seropositive children in June 2020 were reported by their parents to be asymptomatic and, therefore, the timing of their infection was not known. Additionally, we only assessed the correlation of N and RBD antibodies with neutralising activity during the first two rounds of testing in June and July 2020 and assumed that this correlation would be retained in round 3 in December 2020, as has been reported by others. Moreover, whilst we focused on the antibody responses to infection in this analysis, cellular immune responses are also likely to play an important role in protection against SARS-CoV-2 re-infection [26]. Finally, this surveillance was undertaken prior to the emergence and rapid spread of the alpha and delta SARS-CoV-2 variants, which have both been associated with increased transmission compared to previously circulating strains [27,28]. We, therefore, cannot comment on the protective effects of prior SARS-CoV-2 infection against reinfection with new variants.

The majority of primary school students and staff retained RBD antibodies, which strongly correlated with neutralising activity, for more than 6 months after SARS-CoV-2 infection. Our findings provide further evidence of a robust and sustained immune response in children following primary SARS-CoV-2 infection. Further studies are needed to assess protection against emerging variants of concern.

Author contributions

SNL, FB, JB, IO, SA, JG, AB, BB, GA, VS, JLB, KB and MR were responsible for conceptualization and study design/methodology. SNL, FB, JB, IO, SA, JG, AB, BB, GI, FA, ZAC, LL, JF, SEIJ, RB, EL, MZ, AJS, KH, JP contributed to project administration (including laboratory colleagues). SNL, AJS, MZ and GI contributed to the original draft and GI and SNL conducted the formal analysis and were responsible for data validation. All authors contributed to reviewing and editing the manuscripts.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2021.101150.

References

[1] Office for National Statistics. Deaths registered weekly in england and wales by age and sex: Covid-19. Office for National Statistics; 2021 https://www.ons.gov.uk/datasets/weekly-deaths-age-sex/editions/covid-19/versions/26 (accessed 19 April 2021.
[2] World Health Organisation. WHO Coronavirus (COVID-19) dashboard. World Health Organisation; 2020 https://covid19.who.int/ (accessed 19 April 2021 2021).
[3] Buitrago-Garcia D, Egli-Gany D, Counotte MJ, et al. Occurrence and transmission potential of asymptomatic and presymptomatic SARS-CoV-2 infections: a living systematic review and meta-analysis. PLoS Med 2020;17(9):e1003346.
[4] Byambasuren O, Cardona M, Bell K, Clark J, McLaws M, Glasziou P. Estimating the extent of asymptomatic COVID-19 and its potential for community transmission: systematic review and meta-analysis. JAMMI 2020;5(4):223−34.
[5] Addetia A, Crawford KH, Dingens A, et al. Neutralizing antibodies correlate with protection from SARS-CoV-2 in humans during a fishery vessel outbreak with high attack rate. J Clin Microbiol 2020;58(11):e02107−20. doi: 10.1128/JCM.02107-20.
[6] Public Health England. Evaluation of the abbott SARS-CoV-2 IgG for the detection of anti-SARSCoV-2 antibodies. Public Health England; 2020.
[7] Ladhani SN, Baawuah F, Beckmann J, et al. SARS-CoV-2 infection and transmission in primary schools in England in June-December 2020 (sKIDs): an active, prospective surveillance study. Lancet Child Adolesc Health 2021;5(6):417−27. doi: 10.1016/S2352-4642(21)00061-4.
[8] Addetia A, Crawford KHD, Dingens A, et al. Neutralizing antibodies correlate with protection from SARS-CoV-2 in humans during a fishery vessel outbreak with a high attack rate. J Clin Microbiol 2020;58(11):e02107−20. doi: 10.1128/JCM.02107-20.
[9] Lumley SF, Wei J, O'Donnell D, et al. The duration, dynamics and determinants of SARS-CoV-2 antibody responses in individual healthcare workers. Clin Infect Dis 2021;73(3):e699−709. doi: 10.1093/cid/ciab004.
[10] Lumley SF, O'Donnell D, Stoesser NE, et al. Antibody status and incidence of SARS-CoV-2 infection in health care workers. N Engl J Med 2021;384(6):533−40.
[11] Grimwood K, Lambert SB, Ware RS. Endemic non-SARS-CoV-2 human Coronaviruses in a community-based Australian birth cohort. Pediatrics 2020;146(5).

[12] Zimmermann P, Curtis N. Coronavirus infections in children including COVID-19: an overview of the epidemiology, clinical features, diagnosis, treatment and prevention options in children. Pediatr Infect Dis J 2020;39(5):355–68.

[13] Roarty C, Tonry C, McFetridge L, et al. Kinetics and seroprevalence of SARS-CoV-2 antibodies in children. Lancet Infect Dis 2021;21(6):e143. doi: 10.1016/S1473-3099(20)30884-7.

[14] Jeffery-Smith A, Dun-Campbell K, Janarthanan R, et al. Infection and transmission of SARS-CoV-2 in London care homes reporting no cases or outbreaks of COVID-19: prospective observational cohort study, England 2020. Lancet Reg Health Eur 2021;3:100038.

[15] Ladhani SN, Jeffery-Smith A, Patel M, et al. High prevalence of SARS-CoV-2 antibodies in care homes affected by COVID-19: prospective cohort study, England. EClinicalMedicine 2020;28:100597.

[16] Weisberg SP, Connors TJ, Zhu Y, et al. Distinct antibody responses to SARS-CoV-2 in children and adults across the COVID-19 clinical spectrum. Nat Immunol 2021;22(1):25–31.

[17] Yang HS, Costa V, Racine-Brzostek SE, et al. Association of age with SARS-CoV-2 antibody response. JAMA Netw Open 2021;4(3):e214302.

[18] Harris RJ, Whitaker HJ, Andrews NJ, et al. Serological surveillance of SARS-CoV-2: six-month trends and antibody response in a cohort of public health workers. J Infect 2021;82(5):162–9. doi: 10.1016/j.jinf.2021.03.015.

[19] Public Health England (PHE). Evaluation of the abbott SARS-CoV-2 IgG for the detection of anti-SARS-CoV-2 antibodies. Public Health England (PHE); 2020. 08 June https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/890566/Evaluation_of_Abbott_SARS_CoV_2_IgG_PHE.pdf.

[20] Fenwick C, Croxatto A, Coste AT, et al. Changes in SARS-CoV-2 spike versus nucleoprotein antibody responses impact the estimates of infections in population-based seroprevalence studies. J Virol 2021;95:e01828-20. doi: 10.1128/JVI.01828-20.

[21] Caddy SL, Vaysburd M, Papa G, et al. Viral nucleoprotein antibodies activate TRIM21 and induce T cell immunity. EMBO J 2021;40(5):e106228.

[22] McAndrews KM, Dowlatshahi DP, Dai J, et al. Heterogeneous antibodies against SARS-CoV-2 spike receptor binding domain and nucleocapsid with implications for COVID-19 immunity. JCI Insight 2020;5(18):e142386. doi: 10.1172/jci.insight.142386.

[23] Perreault J, Tremblay T, Fournier MJ, et al. Waning of SARS-CoV-2 RBD antibodies in longitudinal convalescent plasma samples within 4 months after symptom onset. Blood 2020;136(22):2588–91.

[24] L'Huillier AG, Meyer B, Andrey DO, et al. Antibody persistence in the first 6 months following SARS-CoV-2 infection among hospital workers: a prospective longitudinal study. Clin Microbiol Infect 2021;27(5):784.e1–8. doi: 10.1016/j.cmi.2021.01.005.

[25] Pierce CA, Preston-Hurlburt P, Dai Y, et al. Immune responses to SARS-CoV-2 infection in hospitalized pediatric and adult patients. Sci Transl Med 2020;12 (564).

[26] Zuo J, Dowell AC, Pearce H, et al. Robust SARS-CoV-2-specific T cell immunity is maintained at 6 months following primary infection. Nat Immunol 2021;22 (5):620–6.

[27] Davies NG, Jarvis CI, CMMID COVID-19 Working Group. Increased mortality in community-tested cases of SARS-CoV-2 lineage B.1.1.7. Nature 2021;593:270–4. doi: 10.1038/s41586-021-03426-1.

[28] Davies NG, Abbott S, Barnard RC, et al. Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. Science 2021;372:6538.