Seroprevalence of SARS-CoV-2 antibodies in children: a prospective multicentre cohort study

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ABSTRACT
Background Studies based on molecular testing of oral/nasal swabs underestimate SARS-CoV-2 infection due to issues with test sensitivity, test timing and selection bias. The objective of this study was to report the presence of SARS-CoV-2 antibodies, consistent with previous infection.

Design This multicentre observational cohort study, conducted between 16 April to 3 July 2020 at 9 UK sites, recruited children of healthcare workers, aged 2–15 years. Participants provided blood samples for SARS-CoV-2 antibody testing and data were gathered regarding unwell contacts and symptoms.

Results 1007 participants were enrolled, and 992 were included in the final analysis. The median age of participants was 10–1 years. There were 68 (6.9%) participants with positive SARS-CoV-2 antibody tests indicative of previous SARS-CoV-2 infection. Of these, 34/68 (50%) reported no symptoms prior to testing. The presence of antibodies and the mean antibody titre was not influenced by age. Following multivariable analysis four independent variables were identified as significantly associated with SARS-CoV-2 seropositivity: known infected household contact OR=10.9 (95% CI 6.1 to 19.6); fatigue OR=16.8 (95% CI 5.5 to 51.9); gastrointestinal symptoms OR=6.6 (95% CI 3.0 to 13.8); and changes in sense of smell or taste OR=10.0 (95% CI 2.4 to 11.4).

Discussion Children demonstrated similar antibody titres in response to SARS-CoV-2 irrespective of age. Fatigue, gastrointestinal symptoms and changes in sense of smell or taste were the symptoms most strongly associated with SARS-CoV-2 antibody positivity.

Trial registration number NCT04347470.

INTRODUCTION
During the first wave of the SARS-CoV-2 pandemic in England, children accounted for just 1% of confirmed infections, 1 had a milder clinical course and had much lower mortality than adults, 1,2 a pattern similar to other international settings. 3 The reasons for this are unknown, but various hypotheses exist. Public health measures, such as school closures, may have minimised children’s exposure to SARS-CoV-2. It is also possible that children have a different immune response to the virus, for example, reduced expression of the ACE2 gene, the host receptor for SARS-CoV-2 virus in airway cells.

Despite existing data, it is impossible to state accurately what proportion of children were infected with SARS-CoV-2 in the UK. Studies based on molecular testing of oral/nasal swabs with real-time reverse transcription PCR (RT-qPCR) underestimate infection due to issues with test sensitivity, timing of testing and selection bias due to only symptomatic individuals undergoing testing. 8 A potentially more reliable method is to test for specific antibodies. Existing antibody tests typically detect IgG or total antibody to either the nucleocapsid or spike proteins of the virus. 9 Antibody testing has partially more reliable method is to test for specific antibodies. Existing antibody tests typically detect IgG or total antibody to either the nucleocapsid or spike proteins of the virus. 9 Antibody testing has greater potential than RT-qPCR to detect previous asymptomatic/mildly symptomatic infection, and is not dependent on coinciding with active infection. Current best seroprevalence estimates from adults in the UK indicate that approximately 6.2% have antibodies consistent with previous SARS-CoV-2

What is already known on this topic?
- Children are relatively unaffected by the SARS-CoV-2 infection with very few requiring hospitalisation.
- A large, but unknown proportion of children with SARS-CoV-2 infection are asymptomatic.
- Molecular testing of oral/nasal swabs underestimates SARS-CoV-2 infection.

What this study adds?
- Gastrointestinal upset is a relatively common symptom of COVID-19 in children. Adding gastrointestinal upset to the list of symptoms triggering a test in children would improve case-finding.
- Asymptomatic and mildly symptomatic children are capable of developing an antibody response to SARS-CoV-2.
- This study did not find a difference in rates of seropositivity or antibody responses according to age in the children of healthcare workers.
Original research

infection.10 These findings are similar to other domestic and international seroprevalence studies.11–14

It is unclear what proportion of children are asymptomatic and which symptoms are most associated with paediatric SARS-CoV-2 infection. Estimates based on RT-qPCR testing of oral/nasal swabs suggest that cough or fever are the most common symptoms.15–20 However, these studies focus on symptomatic cohorts, introducing selection bias,13–20 which leads to underestimation of the asymptomatic proportion.

The objective of this study was to report the presence, and titres, of SARS-CoV-2 antibodies in healthy children of healthcare workers across the UK and to report the symptomatology of infection including the asymptomatic rate.

METHODS

Study design

This multicentre observational prospective cohort study was designed to determine the seroprevalence of SARS-CoV-2 antibodies in healthy children, and report the symptomatology of infection. This study has been written in conjunction with the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.21 The study protocol has undergone external peer review and is available as an open access publication.22

Setting

Participants were recruited from five UK centres, in the four regions of the UK, between 16 April 2020 and 3 July 2020. The sites included tertiary National Health Service (NHS) hospitals (Belfast, Cardiff, Manchester and Glasgow) and a Public Health England site (London).

Participants

Children of healthcare workers, aged between 2 years and 15 years at the time of recruitment, were eligible to participate. A ‘healthcare worker’ was defined as an NHS employee. Healthcare workers were categorised according to role, including whether that role involved patient facing activities. Approximately 150 non-patient facing staff were included to provide a comparison group, and to improve the generalisability of the results. Participants were identified at each participating NHS organisation using internal intranet advertisements and email circulars. Children were excluded if they were receiving antibiotics, had been admitted to hospital within the last 7 days, were receiving oral immunosuppressive treatment or if ever diagnosed with a malignancy.

Informed consent

Informed consent was obtained, and assent given by children where possible. Participants were free to decline/withdraw consent at any time without providing a reason and without being subject to any resulting detriment.

Assessments and procedures

All children underwent phlebotomy performed by experienced paediatric medical and nursing professionals. Serum and/or plasma were tested for antibodies to SARS-CoV-2, in United Kingdom Accreditation Service accredited laboratories using the following assays, which have been validated for use in adults:13–23

► Nucleocapsid assays (Abbott Architect SARS-CoV-2 IgG and Roche Elecsys anti-SARS-CoV-2 total antibody)
► Spike protein assays (DiaSorin LIAISON SARS CoV-2 S1/ S2 IgG assay)

The Abbott, Roche and DiaSorin assays are highly specific for SARS-CoV-2 antibodies, using the manufacturer’s suggested cut-offs, with specificities of 1.00 (95% CI 0.98 to 1.00), 1.00 (95% CI 0.99 to 1.00) and 0.98 (95% CI 0.96 to 0.99), respectively.21–25 They do, however, have lower sensitivities at 0.94 (95% CI 0.86 to 0.98), 0.84 (95% CI 0.75 to 0.91) and 0.64 (95% CI 0.54 to 0.73), respectively.21–25 A summary of the tests used is provided in table 1.

Table 1 Summary of antibody tests used

<table>
<thead>
<tr>
<th>Name of assay</th>
<th>Target</th>
<th>Units</th>
<th>Cut-off</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott Architect SARS-CoV-2 IgG</td>
<td>Nucleocapsid</td>
<td>Calculated index</td>
<td>S/C</td>
</tr>
<tr>
<td>Roche Elecsys anti-SARS-CoV-2</td>
<td>Nucleocapsid</td>
<td>Cut-off index COI</td>
<td>1.0 COI</td>
</tr>
<tr>
<td>DiaSorin LIAISON SARS CoV-2 S1/S2 IgG assay</td>
<td>Spike protein</td>
<td>Arbitrary units (AU/ml)</td>
<td>15.0 AU/ml</td>
</tr>
</tbody>
</table>

The study was powered to detect a change in seroprevalence of SARS-CoV-2 antibodies at three time points (enrolment, and 2 months and 6 months following enrolment). To achieve this, 675 participants were required (assuming alpha of, 0.05 and beta of 0.2). Allowing for a 30% dropout rate, we aimed to recruit 900 participants from five sites. The data presented in this study reflect only the data collected at enrolment and the study is ongoing.

Sample size justification

The study was powered to detect a change in seroprevalence of SARS-CoV-2 antibodies at three time points (enrolment, and 2 months and 6 months following enrolment). To achieve this, 675 participants were required (assuming alpha of, 0.05 and beta of 0.2). Allowing for a 30% dropout rate, we aimed to recruit 900 participants from five sites. The data presented in this study reflect only the data collected at enrolment and the study is ongoing.

Statistical analysis plan

Variables including sex, age, parent role, symptomatology, household contacts and SARS-CoV-2 antibody prevalence were analysed using descriptive statistics (number and proportion for discrete variables, median and IQR for continuous variables). Seroprevalence rates between sites were compared using Fisher’s exact test and antibody titres were correlated with age using the Kendall’s rank correlation test and mean titres were compared


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between symptomatic and asymptomatic participants using the Wilcoxon rank sum test.

Variables associated with SARS-CoV-2 positivity were analysed using univariate and multivariable analyses to identify predictors of SARS-CoV-2 seropositivity. Initially all possible variables were assessed using univariate analysis with Fisher’s exact testing of categorical data, and the Mann-Whitney U test for continuous data (continuous data were skewed). All variables were then included in a weighted binary multivariable logistic regression model. Participants with incomplete CRFs were excluded from univariate and multivariable analyses. Analysis was conducted in R (R Core Team, 2014).

Patient and public involvement
A patient and public involvement (PPI) group comprising parents and children was convened. The PPI group met virtually and via socially distanced meetings. The group contributed to the design of the study through online surveys and video discussions. They have also contributed to media interviews on national television and the lead young person has coauthored a manuscript outlining their experience of taking part in the study.27

Study registration
This study was registered at https://www.clinicaltrials.gov (trial registration: NCT0434740) on 15 April 2020 (last updated 27 May 2020). At the time of registration no patients had been recruited to the study which opened on 16 April 2020. The end of the study will be the last study visit.

FINDINGS
In total, 1042 potential participants were screened for inclusion, of whom 35 were excluded; 18 were outside the specified age range, 1 met specific exclusion criteria and 16 declined consent. The remaining 1007 children were enrolled, of which 35 were excluded; 18 were outside the specified age range, 1 met specific exclusion criteria and 16 declined consent. The median age of participants was 10·1 years (range 2.03 to 15.99 years), with 484 (49%) aged under 10 years; 509 (51%) were male. The roles of participants’ parents are shown in figure 2. There were 359/992 (36.2%) children of hospital medical staff, 191/992 (19.3%) children of hospital nursing/midwifery staff, 95/992 (9.6%) children of community medical staff, 36/992 (3.6%) children of community nursing staff and 160/992 (16.1%) children of other patient facing staff such as radiographers, physiotherapists and other allied healthcare professionals. There were 151/992 (15.2%) children of non-patient facing staff such as managerial and administrative staff.

There were 68/992 participants with positive SARS-CoV-2 antibodies, giving a seroprevalence of 6.9% (95% CI 5.4% to 8.6%, n=992). Of those with positive SARS-CoV-2 antibody tests, 34/68 (50%) reported no symptoms. The most commonly reported symptoms associated with SARS-CoV-2 seropositivity were fever 21/68 (31%), gastrointestinal symptoms (diarrhoea, vomiting and abdominal cramps) 13/68 (19%) and headache 12/68 (18%). The presence of fever, cough or changes in a sense of smell/taste were recorded in 26/68 (38%) of participants. No children within this cohort had severe disease requiring hospital admission. A summary of reported symptoms and their frequency can be seen in table 3.

Seroprevalence of SARS-CoV-2 antibodies varied between sites. Belfast had significantly lower seroprevalence than all other sites at 0.9% (95% CI 0.2% to 3.3%, n=215); p<0.0001, and in London seroprevalence was significantly higher than all other sites at 11.6% (95% CI 7.8% to 16.8%, n=199); p=0.0069. The remaining three sites reported seroprevalence rates between 5.6% and 8.9%. The differences between these three sites were not statistically significant (table 2).

The mean antibody titres, for those testing positive, were; ▶ 4.86 Calculated Index S/C (95% CI 4.28 to 5.45, n=58) for the Abbott Architect SARS-CoV-2 IgG assay.
▶ 65.32 Cut-off Index COI (95% CI 43.24 to 87.40, n=31) for the Roche Elecsys anti-SARS-CoV-2 total antibody assay.
▶ 64.17 AU/ml (95% CI 37.99 to 90.36, n=31) for the DiaSorin LIAISON SARS CoV-2 SI/52 IgG assay.

There was no correlation between age and antibody titres (figure 3). The results from the Abbott Architect SARS-CoV-2 IgG assay indicated a small but significant difference in mean antibody titres between asymptomatic 4.3 S/C (95% CI 3.4 to 5.2) and symptomatic participants 5.5 S/C (95% CI 4.7 to 6.2); p=0.04. There was no significant difference in mean antibody titres for the Roche Elecsys or DiaSorin LIAISON assays when comparing symptomatic and asymptomatic participants (p=0.23 and 0.58, respectively) (figure 3). A table of concordance between the three assays used is available in the online supplemental material.

The univariate analysis of individual variables associated with SARS-CoV-2 seropositivity is shown in table 3. In addition to clinical features, variables such as age, gender, the role of the parent (patient facing or not) and known household contacts

<table>
<thead>
<tr>
<th>Site</th>
<th>Screened</th>
<th>Included participants</th>
<th>Antibody positive</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belfast</td>
<td>217</td>
<td>215</td>
<td>2</td>
<td>0.9 (0.2 to 3.3)</td>
</tr>
<tr>
<td>Cardiff</td>
<td>192</td>
<td>178</td>
<td>10</td>
<td>5.6 (3.1 to 10.0)</td>
</tr>
<tr>
<td>Glasgow</td>
<td>229</td>
<td>224</td>
<td>20</td>
<td>8.9 (5.9 to 13.4)</td>
</tr>
<tr>
<td>London</td>
<td>215</td>
<td>199</td>
<td>23</td>
<td>11.6 (7.8 to 16.8)</td>
</tr>
<tr>
<td>Manchester</td>
<td>189</td>
<td>176</td>
<td>13</td>
<td>7.4 (4.4 to 12.2)</td>
</tr>
<tr>
<td>Total</td>
<td>1042</td>
<td>992</td>
<td>68</td>
<td>6.9 (5.4 to 8.6)</td>
</tr>
</tbody>
</table>

Table 2 Recruitment summary of children of healthcare workers and seroprevalence by site (N and (%) unless otherwise stated)
were included. Age and gender were not significantly associated with SARS-CoV-2 seropositivity (table 3). Parental role showed significant association in the univariate analysis, but this was no longer significant once corrected for site and other variables in the multivariable analysis. Contact with a household member with confirmed SARS-CoV-2 infection was significantly associated with SARS-CoV-2 seropositivity in the participant in both the univariate and multivariable analyses (table 3). The multivariable analysis identified four variables independently associated with SARS-CoV-2 seropositivity: (1) Known household contact with confirmed SARS-CoV-2 ($p<0.0001$), (2) Fatigue ($p=0.001$), (3) Gastrointestinal symptoms ($p=0.0001$), and (4) Changes in sense of smell or taste ($p<0.0012$).

**INTERPRETATION**

This observational study is one of the largest UK studies of paediatric SARS-CoV-2 antibody seroprevalence, and the only study to recruit from all regions of the UK. Following the first pandemic wave in the UK, 68/992 (6.9%) children of healthcare workers had evidence of prior infection with SARS-CoV-2. While this is likely to be higher than the general population it is surprisingly similar to the seroprevalence reported by the Office for National Statistics study of adults from England and Wales (6.2%), and similar to international estimates. As expected, there was marked geographical variation, with London reporting the highest seropositivity rates (11.6%) and Belfast the lowest.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Complete data N (%)</th>
<th>Without SARS-CoV-2 antibodies N (%)</th>
<th>With SARS-CoV-2 antibodies N (%)</th>
<th>OR (95% CI)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (years)</td>
<td>992 (100)</td>
<td>10.1 (5.8)</td>
<td>10.2 (6.9)</td>
<td>–</td>
<td>0.481</td>
</tr>
<tr>
<td>Aged 10 years and over</td>
<td>992 (100)</td>
<td>472 (51)</td>
<td>36 (53)</td>
<td>1.1 (0.6 to 1.8)</td>
<td>0.802</td>
</tr>
<tr>
<td>Male gender</td>
<td>991 (99.9)</td>
<td>468 (51)</td>
<td>41 (60)</td>
<td>1.5 (0.9 to 2.5)</td>
<td>0.133</td>
</tr>
<tr>
<td>Parents (patient contact)</td>
<td>992 (100)</td>
<td>789 (85)</td>
<td>52 (76)</td>
<td>0.6 (0.3 to 1.1)</td>
<td>0.055</td>
</tr>
<tr>
<td>Confirmed household contact</td>
<td>960 (97)</td>
<td>63 (7)</td>
<td>30 (44)</td>
<td>10.9 (6.1 to 19.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fever</td>
<td>962 (97)</td>
<td>102 (11)</td>
<td>21 (31)</td>
<td>3.5 (1.9 to 6.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gastrointestinal symptoms</td>
<td>962 (97)</td>
<td>31 (3)</td>
<td>13 (19)</td>
<td>6.6 (3.0 to 13.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Headache</td>
<td>962 (97)</td>
<td>34 (4)</td>
<td>12 (18)</td>
<td>5.4 (2.4 to 11.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lethargy/fatigue</td>
<td>962 (97)</td>
<td>8 (1)</td>
<td>9 (13)</td>
<td>16.8 (5.5 to 51.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cough</td>
<td>962 (97)</td>
<td>90 (10)</td>
<td>7 (10)</td>
<td>1.03 (0.38 to 2.3)</td>
<td>1.000</td>
</tr>
<tr>
<td>Change in sense of smell/taste</td>
<td>962 (97)</td>
<td>7 (1)</td>
<td>5 (7)</td>
<td>10.0 (2.4 to 37.8)</td>
<td>&lt;0.0008</td>
</tr>
<tr>
<td>Myalgia/arthritis</td>
<td>962 (97)</td>
<td>21 (2)</td>
<td>5 (7)</td>
<td>3.3 (0.94 to 9.4)</td>
<td>0.031</td>
</tr>
<tr>
<td>Sore throat</td>
<td>962 (97)</td>
<td>41 (5)</td>
<td>5 (7)</td>
<td>1.7 (0.5 to 4.4)</td>
<td>0.367</td>
</tr>
<tr>
<td>Shortness of breath</td>
<td>962 (97)</td>
<td>13 (1)</td>
<td>3 (4)</td>
<td>3.1 (0.6 to 11.8)</td>
<td>0.098</td>
</tr>
<tr>
<td>Coryza</td>
<td>962 (97)</td>
<td>27 (3)</td>
<td>1 (1)</td>
<td>0.5 (0.0 to 3.0)</td>
<td>0.715</td>
</tr>
<tr>
<td>Rash</td>
<td>962 (97)</td>
<td>10 (1)</td>
<td>1 (1)</td>
<td>1.3 (0.0 to 9.5)</td>
<td>0.556</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>962 (97)</td>
<td>1 (0)</td>
<td>0 (0)</td>
<td>0.0 (0.0 to 50.8)</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Red type denotes those values that are statistically significant.
Original research

In this study there was a near equal number of children under 10 years of age 32/68 (47%) and children over 10 years of age 36/68 (53%) developing antibodies consistent with previous SARS-CoV-2 infection. Age, as a categorical or continuous variable, was not a statistically significant factor in predicting the presence of antibodies, or the overall titres in children irrespective of the assay used (figure 3).

Of the 68 participants with positive antibody tests, 34/68 (50%) reported no symptoms. The most commonly reported symptoms associated with SARS-CoV-2 seropositivity were fever (21/68, 30%) and gastrointestinal symptoms (13/68, 19%). These symptoms, in addition to fatigue, and changes in sense of smell or taste, were independently associated with previous SARS-CoV-2 seropositivity based on the weighted binary multivariable regression modelling. These findings reflect a number of international studies.15–20 Current UK testing strategies directing testing only for those with fever, cough or changes in smell/taste would have identified 26/34 (76%) of symptomatic participants in this study (assuming 100% sensitivity and specificity of RT-qPCR swab testing). Adding gastrointestinal symptoms would have identified nearly all symptomatic cases in this cohort (33/34, 97%). It is, however, important to note that the predictive value of individual symptoms is context-dependent and their utility will vary depending on the season and the symptomatology of other circulating infections. These findings may be useful to policy makers when considering the best approach to screening paediatric populations for SARS-CoV-2.

There is evidence from adult serological studies that those with severe illness develop a significantly greater antibody response than those with mild or asymptomatic disease.28–30 This has raised concerns that children, who typically have mild disease, may fail to develop a meaningful antibody response to SARS-CoV-2 infection. More recently, emerging adult data suggest that even asymptomatic adults are capable of mounting a potentially lasting and protective immune response.31 32 In our study antibody titres measured using the Abbott Architect SARS-CoV-2 IgG assay were significantly higher in asymptomatic children compared with asymptomatic children (p=0.04). These findings were not replicated with either the Roche Elecsys anti-SARS-CoV-2 or Diasorin LIAISON SARS CoV-2 S1/S2 IgG assays. It therefore remains unclear to what extent the severity of symptoms in children influences the antibody response.

**STRENGTHS/LIMITATIONS**

The strengths of this study are that it is a large multicentre study including children from across the four nations of the UK. The findings are based on systematically screening children for SARS-CoV-2 antibodies and this removes selection bias from assessment of the asymptomatic proportion and determination of symptomatology.

The limitations of this study are:

► The SARS-CoV-2 antibody tests have not been validated for use in children.
► The absolute sample size of seropositive participants is relatively small.
► There was selection bias towards children of hospital staff and children with only mild disease.
► There is a risk of recall bias due to the retrospective nature of data collection relating to symptomatology.

**SUMMARY**

This study demonstrates that approximately half of children with positive antibody tests for SARS-CoV-2 reported no symptoms. This study also demonstrates that younger children were just as likely to have SARS-CoV-2 antibodies as older children and that they are capable of mounting a similar antibody response.
Correction notice This paper has been amended since it was published online. The end of the abstract originally referred to SARS-CoV-1 instead of SARS-CoV-2.

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Contributors TW coordinated the running of the study including data management and processing, and led the PPI group. SC, KF, SF, JE, SA and SL were site leads. CT, AM contributed to the design of the study. PM coordinated and led the PPI group. Isabella Cawley, Emma McManus, Christian Bennison, Kate Mullan, Diarmuid McLaughlin, Christine Maney @julieannmaney, Peter Cawley, Kirsty Gray, Jaclyn Keightley, Sean Kelly, Stephanie Christie, James McKenna, Peter Mallet, Ryan Christie, Katherine Christie, Emma McManus, Christian Bemm, Kate Mullan, Diamuid McLaughlin, Christine Sloane, Rhiamm McKay, Ben Moore, Aleksandra Metyka, Julie Atken, Hayley-Isabella Cawley, Peter Cawley, Kirsty Gray, Jaclyn Keightley, Sean Kelly, Stephanie McCaillan, Mc Nicholus, Fiona Roe, Fiona Russell, Aoife Ryan, Kathryn Smith, Jenna Stewart, Andrew Trester, Z. Morrison, D.Kelly, H. Davies, A. Moore, S. Goyal, B. Saunders, L. Price, P. Robb, S. Richards, H. Davies, T. Cromarty, J. Davies, C. Rees, C. Crosland, L. Power-Kruppa.

Data availability statement Data are available in a public, open access repository. All of the individual participant data collected during this study will be made freely available for research purposes 3 months of completion of the study.

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