Research Paper

High prevalence of SARS-CoV-2 antibodies in care homes affected by COVID-19: Prospective cohort study, England

Shamez N Ladhani, Anna Jeffery-Smith, Monika Patel, Roshni Janarthanan, Jonathan Folk, Emma Crawley-Bovey, Amoolya Vusirika, Elena Fernandez Ruiz De Olano, Marina Sanchez Perez, Suzanne Tang, Kate Dun-Campbell, Edward Wynne-Evans, Anita Bell, Bharat Patel, Zahin Amin-Chowdhury, Felicity Aiano, Karthik Paranthaman, Thomas Ma, Maria Saavedra-Campos, Joanna Ellis, Meera Chand, Kevin Brown, Mary E. Ramsay, Susan Hopkins, Nandini Shetty, J. Yimmy Chow, Robin Gopal, Maria Zambon

* Corresponding author at: Immunisation and Countermeasures Division, Public Health England, 61 Colindale Avenue, London NW9 5EQ, UK.

E-mail addresses: shamez.ladhani@phe.gov.uk, drshamez@aol.com (S.N. Ladhani).

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Introduction

Nursing and residential homes have been disproportionately affected by COVID-19 with high rates of hospitalisations and deaths among residents [1]. In England, the first cases of imported COVID-19 were confirmed in late January 2020 with autochthonous transmission established by early March 2020. Cases peaked in mid-April before declining as a consequence of intense control measures [2]. London, England, was one of the most affected cities and large outbreaks associated with high case fatality rates (CFR) among residents were reported in London care homes [2].
In six London care homes experiencing a large outbreak of COVID-19, 95–100% of staff and surviving residents who had initially tested positive for SARS-CoV-2 RT-PCR RNA on nasal swab had detectable SARS-CoV-2 antibodies five weeks later. Overall, more than two-thirds of residents and staff members had detectable antibodies against SARS-CoV-2 irrespective of their nasal swab results or symptom status. Neutralising antibodies were present in 89% of seropositive individuals and were not associated with age, sex, initial nasal swab positivity, presence of symptoms or resident/staff status.

### RT-PCR testing for SARS-CoV-2

RT-PCR testing for SARS-CoV-2 significantly underestimates the true extent of an outbreak in institutional settings. SARS-CoV-2 seropositivity rates in the care homes affected by COVID-19 were far higher than any healthcare setting, including hospitals, possibly because of the intensity and duration of exposure to the virus within the care home setting. Surveillance is on-going to determine whether SARS-CoV-2 antibodies protect against re-infection and, if so, the duration of protection.

Between 10–13 April 2020, we investigated six London care homes reporting a suspected or confirmed COVID-19 outbreak to Public Health England (PHE) [3]. We found that 40% of residents (105/264) and 21% of staff (53/254) had confirmed SARS-CoV-2, with half of both groups remaining asymptomatic throughout the surveillance period [3]. Mass serological testing can help uncover the true extent of an outbreak in institutional settings. Surveillance is on-going to determine whether SARS-CoV-2 antibodies protect against re-infection and, if so, the duration of protection.

### Methods

We identified six care homes reporting a suspected outbreak (≥2 suspected cases) of COVID-19 to PHE during 10–13 April 2020 [3]. These were nursing or mixed nursing/residential homes of different sizes, providing care for 43–100 residents with 14–130 staff. The care homes were in different stages of a COVID-19 outbreak. During the initial investigation, nasal swabs were taken for SARS-CoV-2 RT-PCR for all residents and staff working in the care home at the time. Infection control measures were reinforced and all SARS-CoV-2 RT-PCR positive individuals were isolated. All tested participants were followed up for any symptoms during the two weeks before, at the time of testing and for two weeks after the test [3].

Follow-up investigation involved a repeat nasal swab and a blood sample from all participants five weeks after the initial RT-PCR testing. The follow-up investigation protocol was reviewed and approved by PHE Research Ethics and Governance Group and participating care homes. Care home managers obtained verbal consent from staff members and from residents who could give their own consent. Otherwise, their next of kin was contacted with information about the additional testing and asked to provide verbal consent over the phone. Testing began on the week of May 18, 2020. Care home staff took nasal swabs for residents and submitted their own samples by self-swabbing with appropriate instructions. Care home nurses took blood samples from residents and their colleagues, with external phlebotomists assisting two care homes with sampling.

### SARS-CoV-2 antibody testing

SARS-CoV-2 infected virus lysate assay: Native virus antigen ELISA was modified from a previously described MERS-CoV assay [7,8]. Microplate bound detergent (Triton X100) extracted lysates of SARS-CoV-2 (isolate England/02/2020) infected Vero E6 cells and uninfected cells were reacted with a serial dilution of convalescent serum obtained from participants in an indirect ELISA format. Virus lysates contained a mixture of viral proteins expressed in Vero E6 cells, including viral nucleocapsid and spike proteins. The reactivity of given sera against virus infected cells and uninfected cells was compared creating a single index value. Sensitivity was determined using convalescent serum samples from SARS-CoV-2 RT-PCR positive individuals at least 21 days after the positive respiratory tract swab [9].

### Microneutralisation assay and neutralising antibody titre

SARS-CoV-2 (isolate England/02/2020) specific neutralising antibody levels were measured using a modification of the WHO influenza microneutralisation methodology [10]. Briefly, 200 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 nucleoprotein (NP) expression determined by ELISA using Rabbit polyclonal to SARS-CoV Nucleoprotein (SinoBiological; Cat no 40143-T62).

The virus neutralising antibody titre was determined as the serum concentration that inhibited 50% SARS-CoV-2 NP expression. All work was undertaken in a BSL-3 laboratory.

### SARS-CoV-2 PCR

Nucleic acid was extracted from samples and analysed by a real-time RT-PCR assay targeting a conserved region of the open reading frame 1ab (ORF1ab) gene of SARS-CoV-2, together with detection of an assay internal control to monitor the extraction and RT-PCR processes. This assay used the primers and probe sequences made public by CDC China (http://ivdc.chinacdc.cn/kyjz/202001/t20200121_211337.html) and required 5μL RNA in a total RT-PCR reaction volume of 25μL. Reverse transcription and PCR amplification was performed on an Applied Biosystems 7500 FAST system.

### Statistical analysis

Descriptive analyses were performed. Data that did not follow a normal distribution were described as medians with interquartile ranges and compared using the Mann-Whitney U test. Antibody
concentrations were presented as ELISA index values with medians and 95% confidence intervals (95% CI). Antibody concentrations above the index value of 0.5 were considered positive. Median antibody concentrations were compared using Kruskal-Wallis with Dunn’s multiple comparisons test adjustment. Categorical variables were described as proportions and compared using Chi-square or Fisher’s Exact test as appropriate. Data were analysed using Stata version 15.0 (Statcorp, Tx) and GraphPad Prism.

Ethics approval: The research protocol was approved by the PHE Research Ethics and Governance Group (REGG Ref: NR0204, 07 May 2020).

Role of the funding source: This study was funded by Public Health England as part of the COVID-19 response. The authors had sole responsibility for the study design, data collection, data analysis, data interpretation, and writing of the report. The authors are all employed by Public Health England, the study funder, which is a public body — an executive agency of the Department of Health. SNL and MZ had full access to all the data in the study and final responsibility for the decision to submit for publication.

Results

Seropositivity

Of the original 518 residents and staff involved in the initial care home outbreak investigation during 10–13 April 2020, 394 (76.1%) consented for follow-up investigations at median of 36 days (range, 30–45 days) and were tested using the virus lysate antibody assay (Fig. 1). SARS-CoV-2 seropositivity for the cohort was 77.9% (95% CI, 73.6–81.7%; Table 1, Fig. 2a). Seropositivity was associated with being symptomatic and SARS-CoV-2 RT-PCR nasal swab positive at the initial test (Fisher’s exact test; both p < 0.0001), but not with gender or being a resident or staff member (Table 1, Fig. 2).

Residents

Twenty-one of the 264 residents tested in the initial investigations died within two weeks and two others died prior to follow-up testing. Thus, 186 of the remaining 241 residents consented to SARS-CoV-2 antibody testing and 81.2% (151/186) were seropositive. Of the 186 residents for who convalescent serological analysis was available 35 had been symptomatic and RT-PCR positive during the initial testing period; all were SARS-CoV-2 antibody positive (Fig. 1, Fig. 2c). For the residents who were RT-PCR positive but remained asymptomatic throughout the outbreak, 97.0% (32/33) were SARS-CoV-2 antibody positive (Fig. 1, Fig. 2c). Of the 118 residents with convalescent serology who had tested RT-PCR negative initially, seropositivity was 85.2% (23/27) in those who had been symptomatic during the outbreak and 67.0% (61/91) in residents who remained asymptomatic (Fig. 1, Fig. 2c).

Staff

Among the 254 staff members involved in the initial investigation, 208 consented to additional investigations and 75.0% (156/208) were seropositive (Fig. 2a). All of those who were SARS-CoV-2 RT-PCR positive at initial testing and symptomatic during the outbreak were positive for SARS-CoV-2 antibodies (22/22), as were 95.5% (21/22) of RT-PCR positive asymptomatic staff (Fig. 1, Fig. 2c). Of the 164 staff members with convalescent serology who were SARS-CoV-2 RT-PCR negative at the initial test time point 21 experienced COVID-19 compatible symptoms during the follow-up period and 18 (85.7%) were SARS-CoV-2 antibody positive. The remaining 143 SARS-CoV-2 PCR negative staff undergoing serological analysis remained asymptomatic throughout the surveillance period; 95 (66.4%) were SARS-CoV-2 antibody positive (Fig. 1, Fig. 2c).

SARS-CoV-2 antibody seropositivity and index values

There was no association between SARS-CoV-2 seropositivity and age (Chi-square test, P = 0.43, Table 1) (Fig. 3, left panel). Among SARS-CoV-2 antibody positive individuals, there was no significant difference in median index value between the those who had been PCR positive or negative at initial testing (Mann-Whitney U Test P = 0.05; Supplementary figure 1a) or by age (Kruskal Wallis with Dunn’s multiple comparisons test P = 0.07; Fig. 3, right panel). There was no association between median SARS-CoV-2 antibody index
value and symptom status (Mann-Whitney U Test \(P = 0.96\); Supplementary figure 1b), or gender (Mann-Whitney U Test \(P = 0.57\); Supplementary figure 1c). Six individuals had equivocal SARS-CoV-2 antibody index values; all were asymptomatic throughout the surveillance period and were SARS-CoV-2 RT-PCR negative on nasal swabs at both timepoints.

Neutralising antibodies

Neutralising antibodies were detected in 89.4% (118/132) of seropositive individuals. There was no association between the detection of SARS-CoV-2 neutralising antibodies and age (Chi-square test \(P = 0.27\); Fig. 4a) or PCR status (Fisher’s exact test \(P = 0.77\)); data not shown. There was no significant difference in neutralising antibody titre by sex (Mann-Whitney U test \(P = 0.69\); Fig. 4b), or symptom status (Mann-Whitney U test \(P = 0.10\); Fig. 4c). There was a trend toward increasing neutralising antibody titre with age (Kruskal-Wallis test with Dunn’s Multiple comparisons, \(P = 0.40\); Fig. 4d).

Nasal swab RT-PCR

All consenting residents and staff had a repeat nasal swab at the time of convalescent blood sampling. Thirteen residents were SARS-CoV-2 RT-PCR positive on this repeat sample, including 10 who had been SARS-CoV-2 RT-PCR positive at an interval of 36–45 days previously, although SARS-CoV-2 RT-PCR Ct values were significantly lower at follow-up (Supplementary Figure 2). Of these ten, 7 consented to serum sampling and all were seropositive with neutralising antibodies detected in all of those tested (Supplementary Table 1). Three residents became SARS-CoV-2 RT-PCR positive at follow-up and all were seropositive (Fig. 1); all remained asymptomatic. None of the staff who were SARS-CoV-2 RT-PCR positive at the initial visit were positive on repeat testing. A previously SARS-CoV-2 RT-PCR negative staff member who remained asymptomatic throughout the surveillance period became RT-PCR positive on repeat testing, with a Ct value of 35.6; this staff member was seropositive for SARS-CoV-2 antibodies.

Table 1

Demographics of care homes cohort and seropositivity by group; percentage and 95% confidence intervals shown. Statistical analysis using Fisher’s exact test(f) and Chi-square test of proportions(c), \(p\) values as shown.

| Sex          | n % total | Seropositive (n) (%) | 95% CI         |
|--------------|-----------|----------------------|----------------|
| Overall      | 394 100   | 307 77.9              | 73.6 - 81.7    |
| Male         | 95 24.1   | 73 76.8               | 67.4 - 84.2    |
| Female       | 299 75.9  | 234 78.3              | 72.2 - 82.6    |
| Symptom status |          |                      |                |
| All          |           |                       |                |
| Symptomatic  | 105 26.6  | 98 93.3               | 86.9 - 96.7    |
| Asymptomatic | 289 73.4  | 209 72.3              | 66.9 - 77.2    |
| p value(f)   |           | 0.0001                |                |
| Residents    | 186 47.2  | 151 81.2              | 75.0 - 86.1    |
| Staff        | 208 52.8  | 156 75.0              | 68.7 - 80.4    |
| p value(f)   |           | 0.78                  |                |
| Residents    | 186 47.2  | 151 81.2              | 75.0 - 86.1    |
| Staff        | 208 52.8  | 156 75.0              | 68.7 - 80.4    |
| p value(f)   |           | 0.78                  |                |
| Residents    | 186 47.2  | 151 81.2              | 75.0 - 86.1    |
| Staff        | 208 52.8  | 156 75.0              | 68.7 - 80.4    |
| p value(f)   |           | 0.78                  |                |
| 1st PCR +    | 112 28.4  | 110 98.2              | 93.7 - 99.7    |
| 1st PCR -    | 282 71.6  | 197 69.9              | 64.3 - 74.9    |
| p value(f)   |           | <0.0001               |                |
| Residents    | 186 47.2  | 151 81.2              | 75.0 - 86.1    |
| Staff        | 208 52.8  | 156 75.0              | 68.7 - 80.4    |
| p value(f)   |           | 0.78                  |                |
| 1st PCR +    | 112 28.4  | 110 98.2              | 93.7 - 99.7    |
| 1st PCR -    | 282 71.6  | 197 69.9              | 64.3 - 74.9    |
| p value(f)   |           | <0.0001               |                |

Fig. 2. Proportion of indicated study populations with positive (maroon), equivocal (yellow) or negative (green) native viral antigen lysate assay. a) Seroconversion for all cohort and staff and resident sub groups. b) Seroconversion by sex for whole cohort. c) Seroconversion by acute RT-PCR result and presence of symptoms for residents and staff. All N = 394; Staff N = 208; Residents N = 186.
Discussion

In six London care homes experiencing a COVID-19 outbreak at the peak of the pandemic, 81.2% of surviving residents and 75.0% of staff were SARS-CoV-2 antibody positive five weeks after the initial outbreak investigation. These rates are far higher than those reported from any other cohort including frontline healthcare workers managing patients with confirmed COVID-19 in hospitals [11-13]. Almost all residents and staff who were SARS-CoV-2 RT-PCR positive on nasal swab at initial testing developed SARS-CoV-2 antibodies, irrespective of whether they were symptomatic at any point during the outbreak. High seropositivity rates were also observed for symptomatic residents and staff even if they had a previously negative SARS-CoV-2 RT-PCR test. The serological investigation emphasises the extent to which SARS-CoV-2 can penetrate vulnerable communities in closed settings, and the underestimation of exposure through point prevalence estimates using RT-PCR from nasal swabs. The lowest seroprevalence was observed in residents and staff who remained asymptomatic throughout the outbreak and who were SARS-CoV-2 RT-PCR negative on both testing visits but, even in this group, more than two-thirds were positive for SARS-CoV-2 antibodies. In this cohort, SARS-CoV-2 antibody levels were not associated with age, sex, presence of symptoms, PCR-positivity or resident/staff status.

There are now sensitive and specific serological assays, such as the one used in this investigation based on using native viral antigens derived from infected cells [9]. Overall, a recent systematic review estimated that serological tests had 30% sensitivity for SARS-CoV-2 antibodies during the first week after symptom onset, rising to 72% in the second week, 91% in the third week and 96% up to 5 weeks later [14]. The finding that almost all residents and staff with confirmed COVID-19 through nasal swab SARS-CoV-2 RT-PCR irrespective of whether they ever experienced symptoms during the outbreak is reassuring and validates the use of our serological assay as a measure of past exposure. The very high seropositivity rates among care home staff compared to 17-44% of patient-facing healthcare workers is staggering [11,12]. A possible
The lower nasal swab positivity during the initial investigations compared to the antibody results five weeks later reflects the limited sensitivity of the test, the quality of sampling, the stage of infection at the time of testing and the gene targets used by different RT-PCR assays [22]. Some of these limitations could potentially have been mitigated by repeated swabbing at different time points. Finally, a quarter of the residents and staff in the initial investigations were not included in the follow-up, either because it was not possible to get informed consent from the residents or their next of kin, or because the staff were not working at the care home when follow-up investigations were performed.

In conclusion, almost all residents and staff with confirmed SARS-CoV-2 infection had detectable antibodies five weeks later, irrespective of whether they were ever symptomatic or remained asymptomatic throughout the outbreak. Additionally, a high proportion of those who were symptomatic but SARS-CoV-2 RT-PCR negative were also seropositive. SARS-CoV-2 antibody levels were not associated with age, sex, PCR positivity, symptom status or resident/staff status. Our findings demonstrate that older and vulnerable residents are able to mount a robust antibody response to SARS-CoV-2 that is similar to younger healthy staff members. This study highlights the value of serological analysis in addition to RT-PCR screening to understand SARS-CoV-2 exposure in this high-risk population, which is vital for informing winter-planning and vaccine strategies. Further studies are needed to determine whether SARS-CoV-2 antibodies protect against re-infection and, if so, the duration of protection.

Declaration of Competing Interest

The authors have nothing to declare.

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Data sharing statement

The data collected were part of enhanced outbreak investigations duties by Public Health England. There are no additional data to share. SNL and MZ had full access to all the data in the study and final responsibility for the decision to submit for publication.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.eclinm.2020.100597.

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