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Serology study of healthcare workers following a hospital-based outbreak of COVID-19 in North West Tasmania, Australia, 2020

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Abstract

Introduction

Healthcare facilities are high-risk settings for coronavirus disease 2019 (COVID-19) transmission. Early in the COVID-19 pandemic, the first large healthcare-associated outbreak within Australia occurred in Tasmania. Several operational research studies were conducted amongst workers from the implicated hospital campus, to learn more about COVID-19 transmission.

Methods

Healthcare workers (HCWs) from the implicated hospital campus were invited to complete an online survey and participate in a serology study. Blood samples for serological testing were collected at approximately 12 weeks (round one) and eight months (round two) after the outbreak. A descriptive analysis was conducted of participant characteristics, serology results, and longevity of antibodies.

Results

There were 261 HCWs in round one, of whom 44 (17%) were polymerase chain reaction (PCR) confirmed outbreak cases; 129 of the 261 (49%) participated in round two, of whom 34 (27%) were outbreak cases. The prevalence of positive antibodies at round one was 15% ($n = 38$) and at round two was 12% ($n = 15$). There were 15 participants (12%) who were seropositive in both rounds, with a further 9% ($n = 12$) of round two participants having equivocal results after previously being seropositive. Six HCWs not identified as cases during the outbreak were seropositive in round one, with three still seropositive in round two. Of those who participated in both rounds, 68% ($n = 88$) were seronegative at both time points.

Discussion

Our findings demonstrate that serological testing after this large healthcare-associated COVID-19 outbreak complemented the findings of earlier diagnostic testing, with evidence of additional infections to those diagnosed when use of PCR testing had been restricted. The results also provide evidence of persisting SARS-CoV-2 antibody response eight months after an outbreak in an unvaccinated population. The high proportion of HCWs who remained seronegative is consistent with low community transmission in Tasmania after this outbreak.

Keywords: COVID-19, seroprevalence, healthcare workers, hospital-based outbreak

Introduction

Healthcare facilities are high-risk settings for coronavirus disease 2019 (COVID-19) transmission, due to the increased risk of healthcare-acquired infections and the significant consequences of transmission within a facility.^{1,2} In April 2020, the North West region of Tasmania experienced a large healthcare-associated outbreak of COVID-19.³ The outbreak was declared on 3 April 2020, one month after the first COVID-19 case had been notified in Tasmania, and when knowledge of transmission of SARS-CoV-2 in healthcare settings was limited. By 29 April, 138 cases were associated with this hospital-based outbreak, including 81 healthcare workers (HCWs) across three hospitals, two geographically located on the same campus. Notably, cases in the outbreak included both clinical and non-clinical staff.³

Initially, during the COVID-19 pandemic in Australia, the capacity for polymerase chain reaction (PCR) testing for SARS-CoV-2 was constrained by shortages of swabs for specimen collection and certain reagents. At the time of the outbreak, guidance restricted PCR testing to symptomatic persons, and therefore PCR testing did not extend to the entire outbreak cohort, nor to asymptomatic contacts. However, given the extent of transmission within the campus, all HCWs from the implicated premises were tested using PCR for return-to-work screening as an additional risk mitigation measure.

As this outbreak was the largest of its kind within Australia at the time, Tasmania's Public Health Services took the opportunity to conduct several operational research studies amongst the cohort of workers from the implicated hospital campus. All clinical and non-clinical staff (including catering, cleaning, and other support services) were invited to complete an online survey to determine the characteristics of those diagnosed with COVID-19, the frequency of potential risk factors for COVID-19, and infection prevention and control practices among HCWs during the outbreak. Respondents were invited to participate in a serology study using

commercially available SARS-CoV-2 immunoassays. These assays had an analytical sensitivity ranging from 92% to 98%, and specificity approaching 100%.⁴ With immunoglobulin G (IgG) seroconversion occurring in 91% to 99% of cases,^{5,6} measuring for the presence of antibodies to SARS-CoV-2 could determine the extent of COVID-19 infection in this cohort, and the longevity of the immune response to natural infection. Some studies had indicated that in the pre-vaccination period, antibodies against SARS-CoV-2 persist for up to six months following natural infection.⁷⁻⁹

The objectives of this study were to describe the prevalence of antibody responses to SARS-CoV-2 and to measure the longevity of SARS-CoV-2 antibodies among the cohort of HCWs who worked at the healthcare campus during the 2020 hospital-based outbreak of COVID-19 in North Tasmania. The study measured antibody prevalence at two time points, approximately 12 weeks ('round one') and eight months ('round two') after the outbreak exposure period. This study was undertaken before COVID-19 vaccination in Australia, which did not commence until February 2021, and when there was no evident community transmission in Tasmania.

Methods

Participants

Participants were recruited from the online survey sent to 1,779 clinical and non-clinical workers who worked on the implicated health campus between 20 March and 13 April 2020. All eligible workers that accessed the online survey could elect to be contacted by the researchers for enrolment into the serology study. For both rounds, participants with positive and equivocal serology results received their result by phone call; participants with negative results received these by email.

Serum collection and testing

The first serum samples (two 5 ml samples) were collected from participants over a six-week

period from 6 July to 14 August 2020, approximately 12 weeks after the outbreak exposure period. The second round of samples were collected between 7 and 24 December 2020, approximately eight months after the outbreak exposure period. Serological testing was performed using Abbott SARS-CoV-2 IgG Assay (Abbott Diagnostics, Abbott Park, IL, USA), a chemiluminescent microparticle immunoassay for qualitative detection of IgG against SARS-CoV-2 spike receptor protein. Results were reported according to the manufacturer's instructions, with a signal/cut off index used for this study as per the manufacturer's instructions (< 0.49: anti-SARS-CoV-2 antibodies not detected; 0.49 to < 1.4: equivocal; and ≥ 1.4 : anti-SARS-CoV-2 antibodies detected).

Data collection and analysis

A purpose-built database was utilised to record the contact details and demographics of participants from the survey who had agreed to be contacted for the serology study, and the results of both rounds of serology tests. Participants who did not complete the survey instead answered five questions about age, sex, occupation, smoking status, and symptom profile during specimen collection. Serology results were linked to participants' responses to either the online survey or the five questions, and to the state-wide SARS-CoV-2 PCR database.

Outbreak cases were defined as participants who had been notified to the Tasmanian Department of Health with a PCR-positive COVID-19 diagnosis during the outbreak period. Outbreak non-cases were participants who had not been diagnosed with COVID-19 during the outbreak period, either because they had a negative PCR test or were not tested during the outbreak period. Positive and negative results of PCR tests performed during the outbreak period and return-to-work testing were reviewed.

A descriptive analysis was conducted of participant characteristics and the serology results, with reference to their outbreak case status. Longevity of antibodies was assessed

by considering the serology results from both rounds as paired outcomes (e.g., seropositive-seropositive, seropositive-seronegative). The number of days between the outbreak and serum sample collections were calculated as the date of each serum collection minus the date of the first positive SARS-CoV-2 PCR result for cases, and the start of return-to-work testing for non-cases (25 April 2020). The analysis was conducted in Stata/SE17 for Windows.

Ethical considerations

Human Research and Ethics Committee approval was obtained through University of Tasmania (Project ID 21786). Informed written consent was obtained from all participants.

Results

Description of participants

There were 261 HCW who provided serum samples in round one; 129 of the 261 from round one (49%) also provided serum samples in round two.

Most participants (84% in both rounds) were female and the median age for participants was 46 years for the first round and 47 years in the second round. Nurses and midwives were the most common occupations (Table 1). There was a higher proportion of outbreak cases in round two compared with round one (27% versus 17%) and compared with those who participated in round one but not round two (8%).

Serology results

Round one

The mean time between the outbreak period and round one specimen collection was 83 days (range: 72–111 days). The prevalence of positive antibodies in this population of HCWs at round one testing was 14.6% (38/261); this increased to a prevalence of detectable antibodies of 19.9% if equivocal results were included (52/261). Of the 38 HCWs with positive antibodies at round one,

Table 1: Characteristics of participants in the serology study, North West Tasmania, 2020

Characteristic	Round one (n = 261) July/August 2020		Round two (n = 129) December 2020		Round one but not round two (n = 132)	
	Number	Percent	Number	Percent	Number	Percent
Sex						
Female	220	84	108	84	112	85
Male	40	15	21	16	19	14
Not reported	1	1	0	0	1	1
Age						
21–30 years	39	15	6	5	33	25
31–40 years	50	19	32	25	18	14
41–50 years	49	19	27	21	22	17
51–60 years	86	33	46	36	40	30
> 60 years	33	13	15	12	17	13
Missing	4	1	1	1	2	1
Occupation^a						
Nurse/midwife	141	54	69	53	72	55
Allied health	28	11	10	8	18	14
Administration	1	0	1	1	0	0
Medical doctor	17	7	11	9	6	5
Cleaner	9	3	3	2	6	5
Catering/food service	2	1	1	1	1	1
Attendant/security	8	3	2	2	6	5
Other	43	16	29	22	14	11
Missing	12	4	3	2	9	7
Outbreak case status						
Outbreak case	44	17	34	27	10	8
Outbreak non-case	217	83	95	73	122	92

a Nurse/midwife occupation includes registered nurse, registered midwife, enrolled nurse, assistant in nursing. Allied health occupation includes dietician/nutritionist, radiographer, occupational therapist, physiotherapist, psychologist, podiatrist, social work, speech pathologist. Other occupation includes phlebotomist, laboratory staff, students.

76% were nurses, midwives, or medical doctors. When the equivocal results were included, this proportion decreased to 72% of 52 HCWs.

Of the 44 outbreak cases, 32 (72.7%) were sero-positive; ten (22.7%) had equivocal serology results; and two (4.5%) were seronegative in the first round of serology testing.

Of the 217 outbreak non-cases, six (2.8%) were seropositive; four (1.8%) had equivocal serology results; and 207 (95.4%) were seronegative. (Table 2).

Of the six outbreak non-cases who were seropositive in the first round of serology testing, only one was tested by PCR during the outbreak period. This case reported having mild symptoms and was tested by PCR twice during the outbreak period and twice after the outbreak period, with all four tests negative. The remaining five outbreak non-cases with positive serology were only tested by PCR after the outbreak exposure period during return-to-work screening. Two participants had a single negative PCR test; one had two negative tests; and two had three negative tests. Two of the five reported no symptoms; two reported mild symptoms; and one reported moderate symptoms during the outbreak. Of these six outbreak non-cases, five were female; the average age was 43.5 years; five were nurses or midwives and one was a doctor.

Round two

The mean time between the outbreak period and round two specimen collection was 230

days (range 219 to 251 days). The prevalence of positive antibodies for the 129 health care workers at round two was 11.6% (15/129); which increased to 22.5% if equivocal results were included (29/129). Of the 15 HCWs with positive antibodies at round two, 73% were nurses, midwives, or medical doctors. When the equivocal results were included, this proportion increased to 76% of 29 HCWs.

Of the 34 outbreak cases in the second round of specimen collection, 12 (35%) were seropositive; 10 (29%) returned equivocal serology results; and 12 (35%) were seronegative. 88 participants (68%) were both outbreak non-cases and seronegative. Three outbreak non-cases remained seropositive, and four outbreak non-cases had equivocal serostatus (Table 2).

Longevity of antibodies

Longevity of antibodies could be considered in the 129 HCWs who participated in both rounds of serological testing. Thirty of the 129 participants in both rounds were seropositive at round 1 (23%), with 15 seropositive in both rounds (50% of those seropositive in round 1 and 12% of the 129 participants). This demonstrates

Table 2: Serology results by outbreak status in round one and two, North West Tasmania, 2020

Round one					
Outbreak case status	Total	Serology result			Mean (range) days between outbreak and round one ^a
		Positive	Equivocal	Negative	
Outbreak cases	44	32	10	2	92 (74–109)
Outbreak non-cases	217	6	4	207	82 (72–111)
Total	261	38	14	209	83 (72–111)
Round two					
Outbreak case	Total	Serology result			Mean (range) days between outbreak and round two ^a
		Positive	Equivocal	Negative	
Outbreak cases	34	12	10	12	240 (219–251)
Outbreak non-cases	95	3	4	88	228 (226–243)
Total	129	15	14	100	230 (219–251)

a Days between the outbreak and serum samples were calculated as the date of the serum collection minus the date of the positive PCR result for cases and the start of return-to-work testing for non-cases (25 April 2021).

persistent seropositivity for an average of 238 days after the outbreak (range: 226–250 days). Twelve of these participants were outbreak cases and three were outbreak non-cases (Table 3).

A further 12 participants were seropositive at round one, an average of 92 days after the outbreak (range: 74–109 days), and then had equivocal serology at round two, an average of 237 days after the outbreak (range: 219–251 days). Ten of these were outbreak cases and two were outbreak non-cases (Table 3).

Three participants, all outbreak cases, were seropositive in round one then seronegative in round two. All seronegative participants in round one were also seronegative in round two. Two outbreak non-cases had equivocal serology results in both rounds. Nine participants (seven outbreak cases and two outbreak non-cases) had equivocal serology in round one and were seronegative in round two (Table 3).

Discussion

In this cohort of 261 HCWs, among whom 44 (17%) were PCR-confirmed outbreak cases during a hospital-wide outbreak, the prevalence of positive antibodies was 14.6% when a first round of serology testing was conducted a mean of 83 days after the outbreak. For the 129 participants in the second round of serology testing, including 34 (26%) who were outbreak cases, the prevalence of positive antibodies was

11.6% when tested a mean of 230 days after the outbreak. The combined prevalence of positive and equivocal serology results was 20% in the first round and 22% in the second. This cohort of HCWs were part of the first and one of the largest outbreaks early in the COVID-19 pandemic in Australia,³ with the seroprevalence of antibodies supporting the clinical and epidemiological findings of a relatively high transmission in this setting. This contrasts with the low seroprevalence found in a 2020 national serology survey, indicative of limited community transmission in Australia at that time.¹⁰

There has been a range of seroprevalences of SARS-CoV-2 antibodies reported among European HCWs during March to September 2020, with many less than 10%.¹¹ Systematic reviews of 28 and 25 studies of HCWs conducted in 2020 found seroprevalences of 7% and 8% respectively;^{12,13} a seroprevalence of 9% among HCWs was reported in a systematic review and meta-analysis of 49 studies in 2021.¹⁴ The seroprevalence in HCWs from this outbreak in Tasmania was higher than many of these studies, but lower than the 27% reported in a cross-sectional study of HCWs from a public hospital in New York City early in 2020,¹⁵ and from studies predominately based in the United Kingdom that reported a seroprevalence between 20% and 45% among HCWs at a similar time.¹¹ Seroprevalence also varied by country, with higher seroprevalence in studies conducted in North America (12.7%) than in those conducted

Table 3: Comparison of paired serology outcomes from round one and two by outbreak status, North West Tasmania, 2020

Paired outcomes		Outbreak cases		Outbreak non-cases		Total	
Round one	Round two	Number	Percent	Number	Percent	Number	Percent
Positive	Positive	12	35	3	3	15	12
Positive	Equivocal	10	29	2	2	12	9
Positive	Negative	3	9	—	—	3	2
Equivocal	Equivocal	—	—	2	2	2	2
Equivocal	Negative	7	21	2	2	9	7
Negative	Negative	2	6	86	91	88	68
Total	Total	34	100	95	100	129	100

in Europe (8.5%), Africa (8.2%) and Asia (4%).¹⁴ Variation in seroprevalence among HCWs may be due to different levels of community transmission; to whether there were outbreaks in healthcare facilities in those countries; and to infection prevention and control practices in these settings.

Our study showed that 22% of the cohort of HCWs were seropositive or had equivocal results an average of 238 days after the outbreak. Detection of anti-SARS-CoV-2 antibodies after natural infection depends on factors including specific assay sensitivity, which ranges from 85 to 97%;^{16,17} and initial antibody response that varies with severity of COVID-19 illness^{18,19} and host factors, such as age.¹⁹ Waning antibody levels after seroconversion to SARS-CoV-2 has been reported from several months after infection²⁰⁻²² to beyond five months.²³ Waning antibody levels were also a notable finding in our study, with seropositivity of outbreak cases halving from 73% at three months to 35% at eight months after infection. Most cases with waning immunity had equivocal serology; several became seronegative.

Almost a third of outbreak cases (27%) had a negative or equivocal serostatus when first tested 12 weeks after the outbreak. Without earlier serology testing, it is unclear whether this is due to a low or absent initial serological response, or to a response then rapid decline in detectable antibodies by the time of the first sample. No individuals seroconverted from equivocal to positive, or from negative to equivocal or positive, between the two tests at three and eight months after the outbreak, reflecting the very low community incidence of COVID-19 in Tasmania at that time.

Our findings highlight the complementary role serology played in COVID-19 diagnosis during this HCW outbreak early in the pandemic, during a time of low transmission and prior to vaccination. It enabled diagnosis of suspected SARS-CoV-2 infection in persons who were PCR-negative and who could not be tested by PCR or antigen testing. Six additional cases

were detected by serology, five of whom were not tested by PCR during the outbreak. The sixth seropositive non-outbreak case tested negative by PCR during the outbreak period. Such discordant results may be due to false negative PCR tests, to the timing of PCR tests, or to a false positive serology test. Infection after the outbreak was unlikely because of the low local prevalence of COVID-19 after the outbreak, but acquisition from within or beyond the affected region cannot be completely excluded. As vaccination had not been introduced to Australia at the time, vaccine-related immunity was not a consideration.

That all seronegative participants in round one were also seronegative in round two supports our understanding that the workplace and community responses to this large outbreak arrested local transmission of COVID-19. Genomic analysis has shown that the genomic sequences linked to this outbreak were contained within the outbreak setting.²⁴ Containment efforts included very restrictive public health and social measures; enhanced infection, prevention and control measures; as well as the unprecedented (at the time) closure of all health facilities on the campus; and mandatory 14-day home quarantine of all HCWs and their household contacts.³ At the time of the outbreak there were state border restrictions, with non-essential travelers required to complete 14 days quarantine upon arrival to Tasmania, and national bans on international travel.

There were several limitations to this study. The small sample size and low response rate from the large cohort of workers from the three implicated healthcare facilities may limit the representativeness of the results. There is also likely to be volunteer bias, with those who volunteered for serology possibly being more likely to be cases or thinking themselves to be cases. This is suggested by the higher proportion of outbreak cases who participated in round two compared with round one. The delay in publishing this study reflects the challenges of

conducting operational research during public health events, especially during a protracted pandemic.

Despite these limitations, this study has shown that retrospective serology testing complemented the restricted PCR testing that was in place during this outbreak investigation. It highlights the usefulness of serological testing performed either contemporaneously or retrospectively, as a complementary diagnostic tool with PCR, to characterise the epidemiology of emerging infections early in settings with little community transmission and in the absence of vaccination. However, for serology testing to be most effective for diagnosis, it would need to be done in real time during an outbreak, which would require considerable resourcing not often available during an emergency response. That this operational research project was undertaken during this outbreak is encouraging, even though subsequent COVID-19 outbreaks throughout Australia overshadowed its relevance and timeliness, including the timeliness to publication. The results of this study provide additional evidence of the longevity of the SARS-CoV-2 antibody response 230 days after an outbreak in an unvaccinated population; the results also demonstrate the quality of the outbreak response by showing the high proportion of persistently seronegative HCWs as evidence that the outbreak was contained without local establishment of community transmission.

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