Seroprevalence of 2009 pandemic influenza A(H1N1) virus in Australian blood donors, October – December 2009

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Assessment of the severity of disease due to the 2009 pandemic influenza A(H1N1) in Australian states and territories has been hampered by the absence of denominator data on population exposure. We compared antibody reactivity to the pandemic virus using haemagglutination inhibition assays performed on plasma specimens taken from healthy adult blood donors (older than 16 years) before and after the influenza pandemic that occurred during the southern hemisphere winter. Pre-influenza season samples (April – May 2009, n=496) were taken from donation collection centres in North Queensland (in Cairns and Townsville); post-outbreak specimens (October – November 2009, n=779) were from donors at seven centres in five states. Using a threshold antibody titre of 40 as a marker of recent infection, we observed an increase in the influenza-seropositive proportion of donors from 12% to 22%, not dissimilar to recent reports of influenza A(H1N1)-specific immunity in adults from the United Kingdom. No significant differences in seroprevalence were observed between Australian states, although the ability to detect minor variations was limited by the sample size. On the basis of these figures and national reporting data, we estimate that approximately 0.23% of all individuals in Australia exposed to the pandemic virus required hospitalisation and 0.01% died. The low seroprevalence reported here suggests that some degree of prior immunity to the virus, perhaps mediated by broadly reactive T-cell responses to conserved influenza viral antigens, limited transmission among adults and thus constrained the pandemic in Australia.

Introduction

The global spread of a novel strain of influenza A(H1N1), which emerged in North America in March 2009, led the World Health Organization (WHO) to declare on 11 June 2009 a phase 6 pandemic alert – the first time in more than 40 years that a pandemic had been declared [1]. Australia's first case of imported active infection with the pandemic virus was reported on 20 May 2009, with confirmation of established community transmission in the state of Victoria only two days later [2]. This timing coincided with the usual onset of seasonal influenza activity, which peaks during the southern hemisphere winter months (June to August) [3]. The epidemic peaked in late July 2009, with most cases reported over an 18-week period until late September, slightly earlier and shorter than a typical influenza season [4]. Influenza-like illness incidence, reported through a variety of sources, appeared similar to that observed in the relatively severe seasonal influenza outbreak of 2007 in Australia [4]. While the majority of reported cases were mild, an excess of hospitalisations and intensive care unit admissions was reported, most markedly in adults aged 20–60 years [4].

Consistent with early observations from other countries [5,6], the pandemic virus appeared particularly transmissible in schools. In Victoria, almost 80% of cases reported during the first two weeks of the outbreak occurred in individuals aged less than 20 years (median: 15 years) [2]. The effective reproduction number (the number of secondary cases per case) only exceeded unity in this younger age group – an effective reproduction number of more than one is a requirement for sustained epidemic growth [2]. Subsequent spread of the virus around the country occurred in a staggered fashion [7], reflecting the large distances between Australian state and territory capital cities, which are mainly dispersed around the coast (Figure 1). Case-reporting rates *per capita* varied over time and by jurisdiction, probably reflecting variable intensity of case-finding efforts by pandemic phase, which was further influenced by local laboratory practices and capacity [7]. While reported hospitalisation and

death rates appeared more similar around the country [7], the absence of a consistent case denominator from which to infer exposure made assessment of severity difficult.

This study aimed to establish a representative collection of plasma samples from healthy adult blood donors in selected Australian jurisdictions following the 2009 influenza A(H1N1) pandemic in the winter. Samples were tested for immunity to the pandemic virus, as a proxy measure of recent virus exposure, to aid assessment of disease severity by age and location. Measurement of the proportion of influenza-seropositive donors would also inform estimates of residual susceptibility to infection in the population, to aid decision-making regarding optimal timing and coverage of proposed population immunisation campaigns.

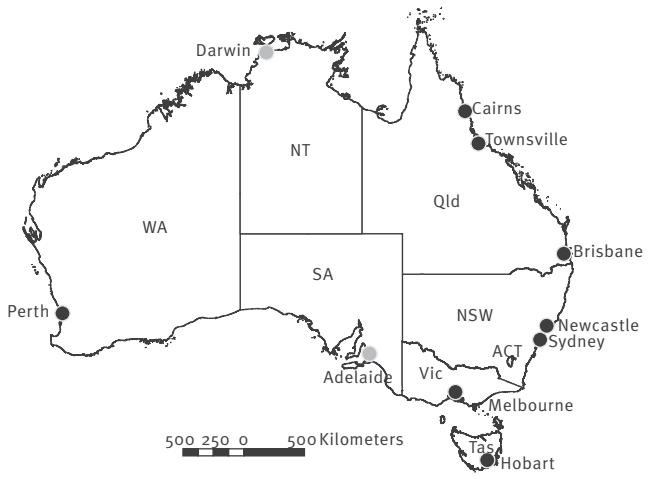
Methods

Pre-pandemic study population

Approximately 500 pre-pandemic plasma samples were randomly selected from anonymised specimens collected in late April – early May 2009 that had been stored by the Australian Red Cross Blood Service (the Blood Service) for dengue fever surveillance studies. The samples were drawn at random from samples stored in a freezer, as were the post-pandemic samples. The sampling time frame was chosen to predate circulation of the pandemic virus in Australia for assessment of baseline immunity. As dengue fever is confined to the tropical north of Australia, such specimens were only available from donor collection centres in Cairns and Townsville, jointly administered through the Townsville site.

Figure 1

Geographical distribution of blood donation collection centres, Australia, April– May and October – December 2009 (n=8)



NSW: New South Wales; NT: Northern Territory; Qld: Queensland; SA: South Australia; Tas: Tasmania; Vic: Victoria; WA: Western Australia.

Blood donation collection centres are marked in black.

Non-participating state and territory capital cities are shown in grey.

Post-pandemic study population

Discarded plasma samples, which had been routinely taken from healthy Blood Service donors for serological testing, were prospectively collected in Brisbane, Hobart, Melbourne, Newcastle, Perth, Sydney and Townsville (Figure 1) from late October to early December 2009 following the first wave of pandemic influenza in Australia [4]. Approximately 120 anonymised specimens were selected per site, with up to 20 randomly selected in each of the following age strata: 16–24, 25–34, 35–44, 45–54, 55–64 and ≥65 years. Accompanying information included age (years) and sex of the donor. Status of prior influenza A(H1N1) disease or vaccination was not routinely obtained at all sites, but most specimens were collected immediately following introduction of the pandemic vaccine, when anecdotally reported uptake was low. Vaccination fields were double checked for participants with high-titre antibodies (>640) to the pandemic virus.

In accordance with the provisions of the National Health and Medical Research Council's National Statement on Ethical Conduct in Human Research, individual consent was not required for use of these specimens, given the granting of institutional approval by the Blood Service Ethics Committee.

Laboratory assays

The tests were performed at the WHO Collaborating Centre for Reference and Research on Influenza, in Melbourne.

Reactivity of plasma against 2009 pandemic influenza A(H1N1) virus was measured using haemagglutination inhibition (HI) assays [8]. Egg-grown A/California/7/2009 virus was purified by sucrose gradient, concentrated and inactivated with β-propiolactone, to create an influenza zonal pool preparation (a gift from CSL Limited). Plasma samples were pretreated with receptor destroying enzyme II (Denka Seiken Co. Ltd), 1:5 (volume/volume) and tested as previously described [9]. Following a one-hour incubation, 25 µl 1% (volume/volume) turkey or human red blood cells (RBC) was added to each well. HI was read after 30 minutes for turkey RBC or 60 minutes for human RBC. Any samples that bound to the RBC in the absence of virus were adsorbed with RBC for one hour and re-assayed. Titres were expressed as the reciprocal of the highest dilution of plasma where haemagglutination was prevented.

The haemagglutinating ability of influenza A viruses can vary depending on the influenza subtype and the species of the RBC used. The ratio of α-2,3- to α-2,6-linked sialic acid residues differs between the RBC of various species [10] and this is reflected in the agglutination patterns of the viral haemagglutinin subtypes [11]. Recent influenza A(H3N2) viruses

typically agglutinate guinea pig RBC better [12], while A(H5N1) viruses agglutinate horse RBC better [13]. The 2009 pandemic influenza A(H1N1) virus agglutinates chicken, human, guinea pig and turkey RBC equally to date (data not shown). Turkey RBC have typically been used in HI assays for 2009 pandemic vaccine serological studies [9,14-16] and are routinely used in the WHO Collaborating Centre in Melbourne and in the other WHO collaborating centres for influenza. Human O-negative RBC are readily obtained and thus routinely used in many research and diagnostic laboratories. For these reasons, we performed HI assays using both turkey and human O-negative RBC.

A subset of samples was also tested by a modified microneutralisation assay [17]. Briefly, undiluted plasma was inactivated at 56 °C for 30 minutes. Heat-treated plasma (two-fold dilutions from 1:10 to 1:1,280) and A/Auckland/1/2009 virus (200 times the 50% tissue culture infective dose $(TCID)_{50}$) were incubated at 35 °C for one hour, then added to washed Madin-Darby canine kidney cells in 96-well flat-bottomed plates, as for $TCID_{50}$ assay, as previously described [18]. Titres were expressed as the reciprocal of the highest dilution of plasma where haemagglutination was prevented.

A panel of control sera and plasma samples was included in all assays. It comprised paired ferret sera pre- and post-infection with the pandemic virus or seasonal influenza A(H1N1), A(H3N2) or influenza B viruses and paired human plasma and sera collected from donors before April 2009 or after known infection with the pandemic virus or after immunisation with the Australian monovalent pandemic 2009 vaccine.

Data on hospitalisations and death

Rates of hospitalisation and death per 100,000 population by Australian jurisdiction were taken from the NetEpi database maintained by the Office of Health Protection, Australian Government Department of Health and Ageing. Permission to use these data was granted by the relevant states and territories that provided the information. The reporting period was from 1 May to 2 October 2009.

Statistical analysis and sample size

Immunity to the pandemic virus was reported as the proportion of donors (by age group or donor site) with HI antibody titres at or above the putative protective threshold of 40 observed to correlate with 50% protection against experimental influenza infection in challenge studies with seasonal influenza viruses, with 95% confidence intervals (CIs) of the estimate [19]. Proportions with HI titres ≥80 and ≥160 were also reported, as well as geometric mean titres (GMT) with 95% CIs. Univariate and multivariate logistic regression models were used to assess the relationship of sex and age of donor with seropositive status (HI titre ≥40) at baseline. In the post-pandemic study population the influence of donor location was also evaluated.

A minimum of 15 individuals within each age stratum was selected as a target sample size to allow estimation of a true seropositive proportion as low as 10%, with 95% confidence intervals excluding 0 and 90% power. A similar protocol involving between 100 and 120 donors of the Red Cross Blood Bank at the Royal Melbourne Hospital was conducted over 14 weeks in 1957. Rising seroprevalence of HI antibodies to the 'Asian' influenza A(H2N2) virus was observed, from 0% to a peak mean value of around 42% by the eighth week of study [20].

Results

Study population

Characteristics of the donor populations from whom samples were collected at each site are shown in Table 1, together with the period of specimen collection. A total of 32 samples were excluded from the analysis due to high haemagglutination titres in the absence of virus and more than two-fold difference between turkey RBC and human RBC. Samples with background HI titres that could not be eliminated by

Table 1

Blood donor characteristics, by collection site and age group, Australia, April – May and October – December 2009 (n=1,307)

Collection site (collection dates in 2009)	Total number of plasma specimens	Blood donor age group (years)						Male (%)
		16–24	25–34	35–44	45–54	55–64	≥65	
Baseline (pre-pandemic)								
Cairns and Townsville, 20 April – 9 May	501	88	59	64	129	132	29	54
Post-pandemic								
Brisbane, 22–30 Oct	107	20	21	16	20	20	10	65
Hobart, 16 Nov – 1 Dec	114	20	21	20	20	19	14	44
Melbourne, 16 Nov	113	20	20	20	20	20	13	54
Newcastle, 24–26 Nov	120	20	20	20	20	20	20	59
Perth, 17–18 Nov	120	20	20	20	20	20	20	50
Sydney, 19–20 Nov	120	20	20	20	20	20	20	65
Townsville, 13–27 Oct	112	19	21	20	20	21	11	63

RBC adsorption were distributed throughout the various locations and age groups. The remaining 1,275 samples were included in the final analysis: 496 from April – May and 779 from October – November 2009.

Assay results

Results are presented for HI assays using turkey RBC (Table 2 and Table 3). A slightly higher rate of background reactivity was seen in the human RBC assays (data not shown), suggesting that a higher threshold titre was required to indicate recent exposure. Despite this difference, there was 90.5% correlation between assay results, with a generally linear relationship ($p<0.001$).

Table 4 reports the findings of a multivariate logistic regression model examining the influence of sex, age and location on post-pandemic seropositivity (HI titre ≥40), although interactions between all factors were not explored due to the limited sample size. At baseline, neither sex nor age had a significant effect on serostatus (data not shown).

Table 2

Haemagglutination inhibition assays of plasma samples from blood donors, by collection site, Australia, April – May and October – December 2009 (n=1,275)

Collection site	Number of samples	GMT (95% CI)	Percentage with HI titre ≥40 (95% CI)	Percentage with HI titre ≥80 (95% CI)	Percentage with HI titre ≥160 (95% CI)
Baseline collection (Apr – May 2009)					
Cairns and Townsville	496	8.40 (7.72–9.14)	12 (9.1–14.9)	6 (3.9–8.1)	3 (1.5–4.5)
Post-pandemic collection (Oct –Nov 2009)					
Brisbane	102	9.03 (7.27–11.2)	18 (10.5–25.5)	9 (3.4–14.6)	6 (1.4–10.6)
Hobart	108	14.6 (10.9–19.5)	31 (22.3–39.7)	23 (15.1–30.9)	12 (5.9–18.1)
Melbourne	107	10.8 (8.40–13.9)	22 (14.2–29.8)	13 (6.6–19.4)	9 (3.5–14.4)
Newcastle	120	10.9 (8.37–14.2)	23 (15.5–30.5)	16 (9.4–22.6)	10 (4.6–15.4)
Perth	117	12.4 (9.63–15.9)	24 (16.3–31.7)	15 (8.5–21.5)	8 (3.1–12.9)
Sydney	116	12.1 (9.53–15.4)	22 (14.5–29.5)	17 (10.2–23.8)	7 (2.4–11.6)
Townsville	109	8.98 (7.25, 11.1)	19 (11.6–26.4)	9 (3.6–14.4)	4 (0.3–7.7)
Total (Oct–Nov)	779	11.1 (10.2–12.2)	22 (19.1–24.9)	15 (12.5–17.5)	8 (6.0–9.9)

CI: confidence interval; GMT: geometric mean titres; HI: haemagglutination inhibition.

In the shaded cells, the post-pandemic HI antibody titres to the 2009 pandemic influenza A(H1N1) virus are significantly higher than those measured in the baseline samples, on the basis of non-overlapping 95% CIs.

Table 3

Haemagglutination inhibition assay results of plasma samples from blood donors, by age, Australia, April – May and October – December 2009 (n=1,275)

Donor age group in years	Number of samples	GMT (95% CI)	Percentage with HI titre ≥40 (95% CI)	Percentage with HI titre ≥80 (95% CI)	Percentage with HI titre ≥160 (95% CI)
Baseline collection (Apr – May 2009)					
16–24	88	9.77 (7.61 to 12.5)	16 (8.3 to 23.7)	9 (3.0 to 15.0)	6 (1.0 to 11.0)
25–34	59	7.72 (6.05 to 9.86)	7 (0.4 to 13.5)	5 (-0.5 to 10.6)	3 (–1.4 to 7.4)
35–44	64	9.07 (7.00 to 11.8)	13 (4.8 to 21.2)	6 (0.2 to 11.8)	5 (–0.3 to 10.3)
45–54	129	7.39 (6.31 to 8.66)	9 (4.1 to 14.0)	6 (1.9 to 10.1)	2 (–0.4 to 4.4)
55–64	129	8.26 (7.10 to 9.61)	13 (7.2 to 18.8)	3 (0.05 to 5.9)	0 (0 to 0)
≥65	27	11.8 (7.64 to 18.2)	19 (4.2 to 33.8)	11 (–0.8 to 22.8)	4 (–3.4 to 11.4)
Post-pandemic collection (Oct – Nov 2009)					
16–24	138	17.1 (13.0 to 22.5)	37 (28.9 to 45,1)	24 (16.9 to 31.1)	16 (9.9 to 22.1)
25–34	139	10.7 (8.75 to 13.0)	22 (15.1 to 28.9)	14 (8.2 to 19.8)	5 (1.4 to 8.6)
35–44	131	9.19 (7.34 to 11.5)	15 (8.9 to 21.1)	13 (7.2 to 18.8)	7 (2.6 to 11.4)
45–54	138	8.56 (7.03 to 10.4)	16 (9.9 to 22.1)	11 (5.8 to 16.2)	5 (1.4 to 8.6)
55–64	131	11.2 (8.86 to 14.1)	20 (13.2 to 26.8)	13 (7.2 to 18.8)	9 (4.1 to 13.9)
≥65	102	12.1 (9.60 to 15.2)	25 (16.6 to 33.4)	11 (4.9 to 17.1)	5 (0.8 to 9.2)

CI: confidence interval; GMT: geometric mean titres; HI: haemagglutination inhibition.

In the shaded cells, the post-pandemic HI antibody titres to the 2009 pandemic influenza A(H1N1) virus are significantly higher than those measured in the baseline samples, on the basis of non-overlapping 95% CIs.

No significant difference in GMT was observed between post-pandemic collection sites, although there was a trend towards a higher seropositive proportion in Hobart than in the other sites (Table 4). Three centres had significantly higher GMTs, demonstrated by non-overlapping 95% CIs, than were measured in the baseline plasma collection (Table 2). Seropositive proportions that differed significantly from baseline are also indicated (shaded cells). The difference between the two collection periods (baseline and post-pandemic) is further demonstrated in the reverse cumulative distribution plots in Figure 2, in which data from October to November specimens have been pooled for all sites, in three age strata.

While titres appeared to rise over time in several age cohorts, the only age group in which GMTs increased significantly from baseline, demonstrated by non-overlapping 95% CIs, was 16–24 years (Table 3 and Figure 2A). The proportion seropositive (HI titre ≥40) rose significantly in both the 16–24 and 25–34 year strata over the course of the outbreak (Table 3), and was significantly higher in the youngest age group than among individuals aged 25–64 years (Table 4, Figure 2B). Figure 2C further demonstrates the higher seropositive proportion in the elderly population at baseline, with very little evidence of exposure resulting in seroconversion during the pandemic. No donors with high-titre antibodies (HI titre >640) had a record of prior immunisation with the pandemic vaccine.

Table 4

Multivariate logistic regression analysis: influence of factors on post-pandemic seropositivity (HI titre ≥40) in blood donors, Australia, October – December 2009 (n=779)

Variable	Odds ratio	95% confidence interval	P value
Sex (reference group: female)			
Male	0.75	0.54–1.05	0.09
Age group in years (reference group: <25 years)			
25–34	0.54	0.32–0.91	0.02
35–44	0.39	0.22–0.68	0.001
45–54	0.36	0.21–0.64	<0.001
55–64	0.58	0.34–0.98	0.04
≥65	0.71	0.41–1.23	0.2
Location (reference site: Brisbane)			
Hobart	1.83	0.99–3.4	0.06
Melbourne	1.23	0.65–2.3	0.5
Newcastle	1.04	0.55–2.0	0.9
Perth	1.22	0.65–2.3	0.5
Sydney	1.16	0.62–2.2	0.6
Townsville	1.01	0.52–1.9	1.0

HI: haemagglutination inhibition.

Correlation between HI and microneutralisation titres, by assay type

A randomly selected subset of 63 samples were further tested by microneutralisation assay for analysis of concordance between the two assay types. For both the turkey and human RBC assays, measured values were higher than observed in the microneutralisation assay. The correlation between assay results was 70.5% ($p<0.001$) with a generally linear relationship observed.

Interpretation of severity, in relation to serosurvey findings

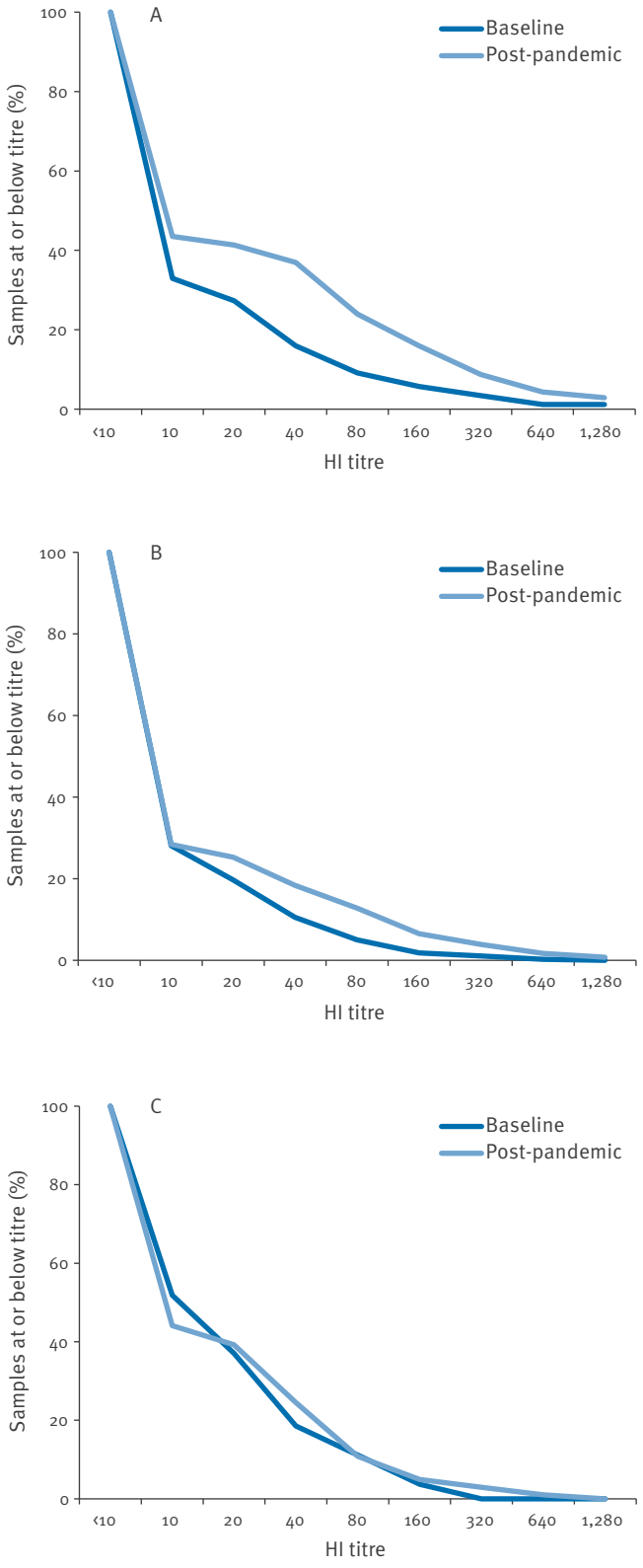
The average reported rate of hospitalisation due to pandemic influenza for the Australian population during the 2009 winter was 23 per 100,000 population [4], ranging from eight per 100,000 population (Victoria) to 40 per 100,000 population (Western Australia). The Northern Territory was a marked outlier, with a rate of 167 per 100,000 population reflecting the heightened susceptibility of indigenous Australians, who make up a greater population proportion in that jurisdiction than elsewhere. Similarly, rates of death due to pandemic influenza in the Northern Territory significantly exceeded those in the rest of the country at 2.7 per 100,000 population, compared with a national average of 0.9 per 100,000 population (range: 0.5 per 100,000 population in Victoria to 1.6 per 100,000 population in South Australia). Assessment of seropositivity in this vulnerable group was not possible as blood donor records do not include indigenous status. In the absence of convincing differences in exposure rates between jurisdictions, the rate of infection exposure sufficient for seroconversion from this serosurvey overall was approximately 10,000 per 100,000 population. On the basis of this figure, we estimate that 0.23% of exposed individuals required hospitalisation and 0.01% died.

Discussion and conclusions

Exposure to the 2009 pandemic influenza A(H1N1) virus appears to have been relatively uncommon among the healthy adult blood donor population during the Australian 2009 winter outbreak. The difference in seropositivity assessed by HI assay compared with baseline was in the order of 5–10% overall, depending on the threshold titre employed for comparison. No significant difference in GMTs was observed between jurisdictions, and only half of the sites surveyed in October – November 2009 had group GMTs higher than in the baseline collection. This finding differs from observations in England and Wales [21] and Scotland [22], suggesting a greater degree of heterogeneity of population mixing in those countries than in Australia. Despite vast geographical distances, the majority of the largely urbanised Australian population is concentrated in a handful of state capital cities, which act as important hubs for each jurisdiction [23]. The only age group in which GMTs were significantly higher (on the basis of non-overlapping 95% CIs) after the winter outbreak was the 16–24-year-old cohort, consistent with trends observed in the United Kingdom (UK) studies [21,22].

Figure 2

Reverse cumulative distribution plot of haemagglutination inhibition titres of plasma samples from blood donors collected Apr – May 2009 (baseline) and Oct – Nov 2009 (post-pandemic), by age group: (A) <25 years, (B) 25–64 years, (C) ≥65 years



HI: haemagglutination inhibition.

This study has a number of limitations that must be taken into account when interpreting its findings. Time and budgetary constraints necessarily limited the number and frequency of specimen collections. As plasma samples for serological testing are routinely discarded after several days in most centres, stored specimens for baseline antibody assessment were only available from Cairns and Townsville, where collections are maintained for research purposes. Given the connectedness of the Australian population, we do not believe that this is likely to have biased baseline assessment of immunity, but a nationwide survey would have been preferable. Also, the collections were cross-sectional in nature and did not allow measurement of evolving immune status within an individual over time, which would provide a more accurate assessment of seroconversion. Moreover, specimens used for this study were recovered plasma samples from blood donors taken after completion of mandatory viral nucleic acid testing. Stringent procedures are in place to ensure that blood donors are healthy at the time of donation, but their past illness experience is unrecorded. Donors might differ from the general population in relation to illness avoidance behaviours as well as in the prevalence of risk factors for infection and severe disease. They were also, by definition, at least 16 years of age, precluding inclusion of paediatric samples.

Higher rates of background reactivity are observed in plasma than in serum; the laboratory methods employed in this study were therefore designed to minimise this additional 'noise'. Plasma samples have previously been used in related studies as part of dengue blood donor surveillance studies for less stable viral RNA markers [24].

HI assay results vary significantly between laboratories, despite best efforts at standardisation [25]. HI titres in this study tended to be higher than microneutralisation titres, as observed in baseline samples assayed in a recent pandemic vaccine trial [15]. Post-immunisation measures of immunity by either assay were more closely concordant [15]. Interpretation of population susceptibility to disease based on these results is made more difficult by the absence of definitive correlates of exposure to or protection against influenza infection. The HI threshold titre of 40 required for seasonal vaccine licensure is based on historical demonstration of 50% protection against experimental infection with partially attenuated challenge strains [26,19]. Household cohort studies suggest that an HI titre of 80 substantially reduces the risk of naturally acquired influenza A(H3N2) infection, with lower titres associated with a modified disease course [27]. Paired serum samples from a limited number of patients (n=10) with known 2009 pandemic influenza showed at least a four-fold rise in HI titres to the pandemic virus between collection times in nine of the patients. Further, all patients who seroconverted had an HI titre of ›40 for their second bleed (unpublished

data, K.L. Laurie). Protection from subsequent infection in patients known to have been infected will be of interest as there are no definitive correlates of recent exposure to a novel influenza viral strain.

Baseline reactivity to the pandemic virus was somewhat higher across the age spectrum in our study than previously observed, but similarly low in people aged over 65 years in estimates from a recently published United States (US) study of vaccine trial participants [28], as well as a UK serosurvey [21]. While the influenza-seropositive proportion was higher in a study of elderly people (aged >65 years) in Finland [29], only 5% of the blood donors we studied exceeded 66 years of age; the eldest participant was aged 78 years. Overall rates of pandemic virus seropositivity in the October–November 2009 samples assessed by GMT or defined by an HI threshold titre of 40 were further similar to those observed in a pandemic vaccine study in adults [15]. That trial was conducted in 240 healthy adult participants aged between 18 and 64 years without prior evidence of pandemic virus infection in Adelaide recruited between 22 and 26 July 2009, around the time of the peak of the pandemic in South Australia [15]. Other estimates of disease severity have reported hospitalisation and death rates in relation to inferred symptomatic case presentations, rather than seroconversions, making direct comparisons difficult [30,31]. While reported influenza hospitalisation rates *per capita* were higher in Australia than the US [30], severity in relation to all estimated infections appeared less because of a greater 'exposure' denominator, perhaps suggesting that a large proportion of cases inferred from our study were asymptomatic.

The apparently low rate of population exposure suggested by this serosurvey might be an underestimate of the true attack rate if first exposure to the pandemic virus was poorly immunogenic, resulting in low and/or rapidly declining antibody responses. Poor immunogenicity of the novel virus seems implausible, however, given the robust immune responses to the pandemic virus as a vaccine antigen after only a single 15 g dose in adults [15] and the data from infected patients discussed above. Furthermore, the figure is very similar to that estimated in New Zealand where pandemic influenza had similar characteristics [32]. Alternatively, the effective reproduction number of the virus in adults may have been substantially lower than that observed in children or overall. Such inference was drawn from observations during the intensive case-finding and management phase during the initial weeks of the pandemic response in Victoria, during which time approximately 80% of reported cases were among children [2]. Over this period, the number of secondary cases per case only exceeded one (an essential requirement for epidemic growth) for transmissions between individuals under the age of 20 years, suggesting significant constraint of infectiousness between adults [2]. These findings were consistent with modelling evaluation of the initial outbreak of pandemic influenza A(H1N1) respiratory infection described in La Gloria, Mexico, where children were estimated to be both substantially more susceptible to and infectious with the pandemic virus than adults [5]. Further, a recent analysis of data from the US on within-household transmission of the pandemic virus has demonstrated that children are twice as likely as adults to be infected by an index case in the family [33].

If the true exposure rate in the population was less than 10%, can we explain how the 2009 pandemic influenza stopped? Given the limited application of social distancing measures, restricted to the early 'contain' response [4], and minimal use of antiviral prophylaxis, one possible explanation is to infer partial protection of the population through antecedent exposure to seasonal viruses [34]. T-cell epitopes in the pandemic virus are highly conserved in relation to recently circulating seasonal influenza viruses [35]. There is strong suggestive evidence of a role for broadly cross-reactive cellular immune responses in reducing morbidity and mortality from seasonal and pandemic influenza infection in humans [36]. Accordingly, one study has demonstrated an inverse correlation in humans between the presence of inducible cytotoxic T-cell responses and virus shedding following experimental influenza infection [37], with likely but untested implications for infectiousness. Even partial reduction of infectiousness among adults by these means would have a substantial impact on transmission at a population level, reducing the effective reproduction number below unity and halting an outbreak more rapidly than may be anticipated from measurement of the proportion seropositive by HI assay alone.

This study suggests that exposure to the pandemic virus during the 2009 winter season was relatively uncommon among the healthy Australian adult population, at around 10%. Further evaluation of specimens from children is required in order to assess ongoing susceptibility to the virus in that more vulnerable age group, in whom transmission potential has been clearly demonstrated. Additional plasma collections prior to and following the 2010 influenza season are envisaged, to aid interpretation of relative exposure and severity of H1N1 infections.

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