What serosurveillance studies tell us about the 2009 influenza pandemic

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Surveillance of the impact of pandemic (H1N1) 2009 influenza during its initial seasons in both hemispheres relied on routinely available indicators, including numbers and rates of laboratory-detected cases, hospitalisations, ICU admissions and deaths, along with monitoring of influenza-like illness (ILI) from primary care sentinel surveillance systems. Estimates of the clinical attack rate and the case fatality ratio were imperfect. Understanding of the pathogenicity of the pandemic virus and prediction of the impact in subsequent seasons was hindered by a lack of information on actual infection rates in the population. Results of a number of serosurveys conducted in Australia and overseas countries have now become available, revealing that the arrival of the pandemic virus in modern urbanised and non-immune populations resulted in relatively similar infection rates in both the southern and northern hemispheres. Around 30–50% of children and teenagers were infected during the first pandemic season, with lower rates, around 10–20%, in young and middle-aged adults, and very few infections in older adults. There were significant numbers of mild or asymptomatic infections, and case fatality and hospitalisation ratios were much lower than those contemplated in pandemic plans. Many populations, including Australia, achieved a significant level of herd immunity during the first wave, and community susceptibility was further reduced by vaccination programs, although coverage was lower than expected. In the absence of significant antigenic drift or changes in virulence, the impact of the pandemic H1N1 virus should continue to decline in future influenza seasons.

The impact of pandemic (H1N1) 2009 influenza (pH1N1)

Following its unexpected emergence in 2009, assessment of the impact of pH1N1 relied on routine or enhanced collection of indicators of disease occurrence and severity, including numbers and rates of laboratory-confirmed infections and hospitalisations, ICU admissions and deaths associated with confirmed infection. This information was supplemented by monitoring of influenza-like illness (ILI) from primary care sentinel surveillance systems, estimates of transmission parameters such as the effective reproduction number (R) and household transmission rates, and imperfect estimates of the clinical attack rate and the case fatality ratio[1-4].

The picture that emerged was of a virus that in general caused relatively mild clinical infections, not dissimilar to seasonal influenza[5], but it was difficult to reconcile apparent conflicts in the surveillance data regarding the impact of the pandemic. On the one hand, there were much higher rates of laboratory-confirmed cases than in inter-pandemic seasons and a heavy burden on intensive care units from those seriously ill with viral pneumonitis, including a significant proportion of young adults without recognised risk factors[1,6,7]. Conversely, hospital emergency department and general practice sentinel systems indicated that rates of ILI in the community were not dissimilar to experience in recent influenza seasons, and influenza-specific and all-cause mortality was not increased[1,7].

It also became apparent within weeks of the emergence of pH1N1 that older adults were relatively protected from infection, as indicated by significantly lower rates of laboratory-confirmed infections and hospitalisations compared to children and young adults[1], and serological evidence of cross-protective antibodies against the pandemic virus, thought to reflect long-ago exposures to 1918-like H1N1 virus strains[8]. It has also been speculated that the lower susceptibility of older adults to the

2009 pandemic virus might reflect heterotypic immunity from anti-neuraminidase antibodies and/or cell-mediated responses against conserved influenza virus epitopes[8,9].

Understanding of the pathogenicity of pH1N1 and forecasting of the likely impact in future seasons was critically hampered by the lack of information on population infection rates from seroepidemiological studies[10]. This piece of the pandemic puzzle is now belatedly being answered, as data become available from serosurveys conducted in a number of populations. A range of study designs have been employed, including: one-off estimates of **seroprevalence** at the end of the first or second pandemic waves[11-14]; serial estimates of prevalence, using independent convenience samples of specimens, mostly residual sera collected for other purposes, from before and following the first and/or second pandemic waves to demonstrate **increasing seroprevalence** and in some instances to estimate cumulative **infection rates**[15-24]; and less frequently, more direct determination of infection rates by demonstrating seroconversion in individuals followed longitudinally over an influenza season[24,25]. In comparing results of these studies, it is important to consider differences in overall study design and more specific aspects of methodology, including the timing of specimen collection in relation to pandemic activity and commencement of vaccination programs, mismatches by age and/or geographic location in pre- and post-pandemic study groups[19], use of different cut-points to define seropositivity, and differences in age distribution and geographic representativeness of study populations.

Measuring influenza infection serologically

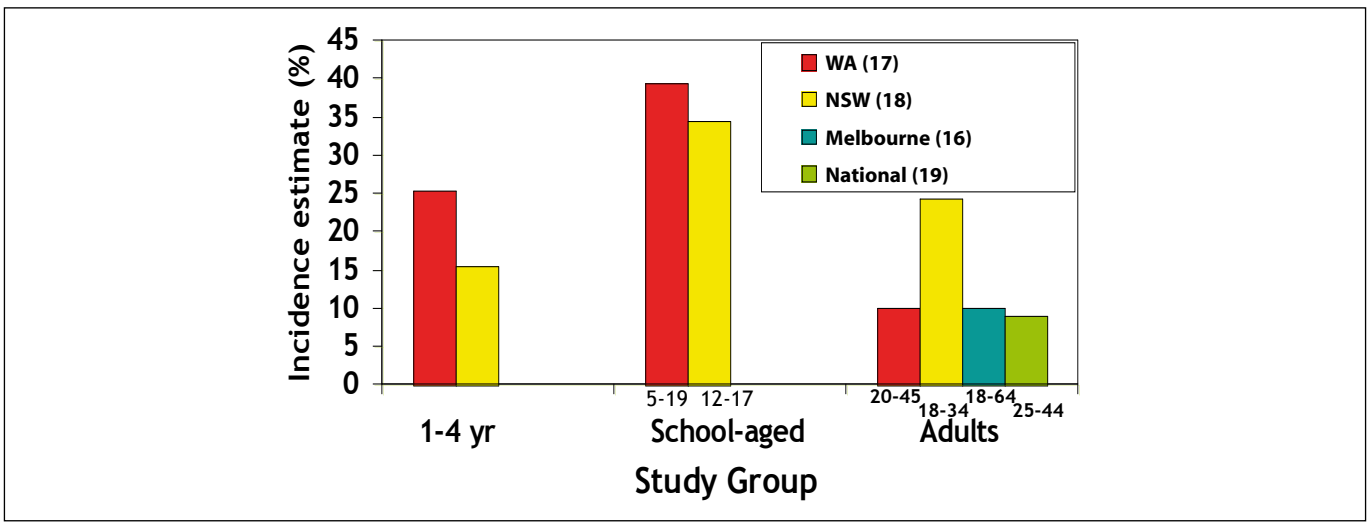
Serological detection of influenza infection in man has traditionally been performed by either haemagglutination inhibition assay (HI) or complement fixation test (CFT) in preference to other methods such as ELISA or the virus neutralisation test (VNT). HI and VNT can determine serological responses to specific viruses or subtypes whereas the CFT test can only distinguish responses to influenza A or B viruses[26]. The sensitivity and throughput of the HI test is generally higher than CFT, so this has been the test of choice for large serosurveys such as those required to determine the attack rate following a pandemic. HI titres of ≥32 or ≥40 are generally taken to define seropositivity[15-20] as these cut-offs have been associated with decreased risk of infection and are used for annual influenza vaccine registration trials[27]. When paired sera (acute and convalescent samples) are available a fourfold rise in HI titre is preferred[25]. For VNT a range of cut-offs have been used according to age (≥40 for children and ≥160 for adults)[8]. Serological testing in the 1957 pandemic using HI demonstrated a rising proportion of people with serological responses following the circulation of the A(H2N2) subtype[28].

Evidence of influenza virus infection as shown by HI seroconversion has been compared to the detection of virus in respiratory samples during active disease using real-time RT-PCR. Use of "significant" HI titres (≥32 or ≥40) may underestimate true infection rates, as suggested by a study which found that 10% of PCR-confirmed pH1N1 cases had HI titres <32 more than three weeks after illness onset[15]. Conversely, an Australian study found that up to one-third of severely ill pH1N1 cases with demonstrated seroconversion had negative PCR tests[29].

Australian serosurveys

In Australia, data from Western Australia (WA)[17], New South Wales (NSW)[18], Victoria[16], the Northern Territory (Trauer, J. *et. al*., personal communication) and a national survey of blood donors[19] indicated high rates of pH1N1 infection in children and relatively low rates in adults (Figure 1). All studies used HI assays, mostly performed in the same laboratory, and antibody titres of ≥40 were taken to indicate significant exposure. In WA, it was estimated that 25% of pre-school children, 39% of school-aged children and older teenagers, and only 10% of pregnant women aged 21–45 years were infected with pH1N1 during the 2009 winter, prior to commencement of the pandemic vaccination program, suggesting high levels of mild or asymptomatic infection in children[17].

Figure 1. Estimated cumulative incidence of pandemic (H1N1) 2009 influenza infection in winter 2009 from Australian seroprevalence studies. (See references for details: HI titres ≥ 40 against pH1N1; pre-pandemic prevalence estimates subtracted from post-pandemic prevalence; age groups shown on horizontal axis).



Data from NSW indicated somewhat lower infection rates in children (16% in pre-schoolers, 20% in 5–17 year-olds), but higher rates in young (24%: 18–34 years) and middle-aged (20%: 35–64 years) adults and effectively no infections in adults aged over 64 years[18]. In Melbourne, which experienced an early intense 2009 winter epidemic, the estimated infection rate in adults aged 18–64 years was 10%[3,16], similar to the overall estimate from the national blood donor study (ages 16–78 years)[19]. The latter study estimated national infection rates of 21% in those aged 16–24 years and 15% in 25–34 year-olds, but once again there was little indication of exposures resulting in seroconversion in older Australians[19].

The WA[17], Melbourne[16] and national[19] studies suggest that seroprevalence and estimated infection rates were relatively homogeneous geographically within and between states, whereas the NSW[18] (Sydney versus rest of NSW) and Northern Territory (Trauer, J. *et. al.*, personal communication) (between areas based on remoteness) studies reported some heterogeneity in infection rates during the 2009 winter season.

International serosurveys

A national New Zealand study estimated pH1N1 infection rates at the end of 2009 for pre-school children (28.2%), school-aged children (34.3%) and adults aged 20–39 years (17.6%) that were similar to the Australian estimates, particularly those from WA[17,20]. More surprisingly, estimates of pH1N1 infection rates for the London and West Midlands regions of England following its initial intense "summer" epidemic wave in 2009 were 21.3% in children <5 years, 42.0% in children aged 5–14 years and 6.2% in adults aged 25–45 years, again remarkably similar to the Western Australian estimates[15,17]. Infection rates estimated at the conclusion of the first wave in Hong Kong were also consistent: 43% in children aged 5–14 years, 16% in 15–19 year-olds, and 8.5% in adults aged 20–39 years[24]. Reassuringly, this study also determined that pH1N1 infection rates estimated from calculating the difference in seroprevalence between pre- and post-pandemic, cross-sectional survey groups were similar to those determined from a smaller group in whom seroconversion was demonstrated[24].

A study in Pittsburgh in the USA[13] measured seroprevalence in November–December 2009 after the peak of their 'second pandemic wave' and following implementation of the pandemic vaccination program, and found age-specific seroprevalences similar to the levels recorded in England[15], WA[17], New Zealand[20] and Hong Kong[24] following their first waves, but before commencement of vaccination. This suggests that infection rates in Pittsburgh, and possibly wider USA, were not initially as high as those experienced during the first waves in England, Hong Kong and the southern hemisphere countries of Australia and New Zealand. In Norway, by contrast, by January 2010 the prevalence of HI titres ≥40 against pH1N1 was as high as 65% in 10–19 year-olds, and 30–40% in older age groups, reflecting both an intense autumn (October–November) epidemic in 2009, and estimated vaccine coverage of 40% of the population[21]. Several studies suggested geographic heterogeneity in the timing and intensity of the initial (summer 2009) and autumn/winter 2009–10 pandemic waves within and between European and other northern hemisphere countries, leading to differences in seroprevalence and estimated infection rates[11,12,15].

In the equatorial international transport hub of Singapore, seroconversion to pH1N1 was determined in individuals from several cohorts studied during its first pandemic wave between May and September 2009. Infection rates were high in young military personnel (44% and 24% in 15–19 and 20–24-year-olds, respectively), but lower in older community members aged 20–39 years (15%) and over 49 years (only 8%)[25]. Despite the different method, these results are broadly similar to the infection rates estimated in studies in Australia and elsewhere using separate pre- and post-pandemic, cross-sectional population samples[15,17,19,20].

There are few serosurvey data available for developing country populations, but a study performed in Pune in India in the latter part of 2009 also found high rates of pH1N1 seropositivity in younger age groups – 27% and 42% in 10–14 and 15–19-year-old school children, respectively, although lower rates were determined in a community sample of 15–19-year-olds (20%) and adults (4%)[22].

Seroprevalence or estimated infection rates for pregnant women, a group that seemed disproportionately at risk of severe outcomes from the pandemic virus[2,6], were determined at the end of the first pandemic waves experienced in WA[17], France[14] and Canada[23]. Each of these studies found infection rates around 10%. This figure is consistent with that found in men and women (not differentiated by pregnancy) of similar age in other studies[15,16,19,20,24], and suggests that higher rates of adverse outcomes in pregnant women reflected a real increase in disease severity, and were not a result of higher infection rates associated with greater exposure to children and/or increased susceptibility to infection *per se*.

Clinical attack rates and case fatality ratios

A meta-analysis of studies of seasonal H1N1 influenza found that around one-third of infected volunteers were asymptomatic, and only 37% were febrile[30]. It seems clear that the 2009 pandemic virus behaves similarly, given the uniformly high infection rates in children estimated from serosurveys in many populations. More directly, studies in Hong Kong[31] and New Zealand[32] found that 36% and 45%, respectively, of individuals with increased or significant HI titres against the pandemic virus were asymptomatic. The latter study estimated that 18.3% of New Zealanders were infected with pH1N1 during 2009, giving a population clinical attack rate of 10%, and that 29.5% of the population had immunity against the virus at the end of 2009. The case fatality and hospitalisation ratios were 8.2 and 262 per 100,000 symptomatic cases, and 4.5 and 144 per 100,000 infected cases, respectively[32]. Using data from the national serosurvey, McVernon *et al.*[19] estimated similar pH1N1 case fatality (10 per 100,000) and hospitalisation (230 per 100,000) ratios for Australian adults infected with the pH1N1 virus. In Hong Kong,

it was estimated that 10.7% of persons aged 5–59 years were infected during the first pandemic wave, with case fatality and hospitalisation ratios of 4.4 and 730 per 100,000 infected cases, respectively, suggesting a lower threshold for hospitalisation than in New Zealand and Australia, but similar case fatality[24]. The estimated hospitalisation and case fatality ratios for pH1N1 from Australia, New Zealand and Hong Kong are well below those contemplated in pandemic plans prepared prior to 2009, which were based on the assumption a pandemic virus would cause more severe outcomes[33].

Conclusions

Despite some regional variation and differences in methodology, serosurveys indicate that infection rates associated with the first wave of the pandemic (H1N1) 2009 influenza virus were broadly similar in modern urbanised and non-immune populations in both the southern and northern hemispheres. Older adults were relatively protected, there were moderate infection rates in young and middle-aged adults of around 10–20%, but children and teenagers experienced infection rates of around 30–50%, at the upper end of rates observed previously for seasonal influenza[34,35]. Similarly to seasonal influenza, a significant proportion of people infected were asymptomatic or had only mild symptoms. Case fatality and hospitalisation ratios were much lower than those contemplated in pandemic plans. Given the important role played by children in transmission of influenza in the community, many populations, including Australia, achieved a significant degree of herd immunity during the first wave, and community susceptibility was further reduced by population vaccination programs in the latter part of 2009 and during 2010, even though vaccine uptake was generally lower than anticipated[36,37]. In the absence of significant antigenic drift or changes in virulence, the impact of the pandemic H1N1 virus should continue to decline in future influenza seasons.

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