

MINIREVIEW

Seroprevalence to Influenza A(H1N1) 2009 Virus—Where Are We?[†]

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Age-specific seroprevalences for influenza virus make important contributions to estimating the burden of infection and determining the vulnerable populations. It is especially difficult to know the true clinical attack rates of the 2009 influenza A(H1N1) pandemic; however, we can estimate infection rates through analyses of seroprevalences based on national studies from different continents and countries with different demographics. After the 2009 influenza A(H1N1) pandemic, seroprevalence studies found 5 to 60% of populations across different continents and age groups having antibodies against the A(H1N1) 2009 virus. The seropositivity was highest in children and teenagers (20 to 60%) as well as in the elderly older than 80 years (20 to 40%). Preexisting cross-reactive antibodies against the virus were present mostly in sera of older people (born before 1950) who could have encountered viruses descended from the 1918 pandemic viruses. Experience with the 2009 pandemic indicates how essential early and timely serology data against the emerging virus can be for informing decisions on use of antivirals and vaccination campaigns, especially in regard to risk groups. The objectives of this review were to summarize the current data available on seroprevalence before and after the 2009 influenza A(H1N1) pandemic and the lessons learned for future pandemic preparedness.

Serology is the identification of antibodies in serum. It is widely used to estimate the true incidence or prevalence of exposure to a suspected pathogen or infection with a pathogen in an individual, in a population, or in cohorts. Conversely, it is also often taken to indicate potential vulnerability. By means of serology, it was possible to assess preexisting levels of likely susceptibility to the recent 2009 influenza A(H1N1) pandemic prior to its start. Later, it was used to estimate the proportions of the populations that were infected in the subsequent waves. This information was important for determining information required for efficiently mitigating the effects of the pandemic (Table 1). Having entered the first postpandemic period in 40 years, this may be a good moment to review the contribution and findings of prevalence in the 2009 pandemic. Prevalence can only be estimated based on serological studies, since the rate of asymptomatic infections cannot be measured directly, an issue that especially arose in the 2009 pandemic with there being many infections that were asymptomatic or with subtle presentations. Furthermore, the care-seeking behavior differs by age of the patient, often being higher in children than in adults.

Influenza remains a major cause of morbidity and mortality globally, with large segments of the population infected every year (17), and therefore, there are several important reasons to collect influenza seroepidemiological data. First, true infection rates can only be estimated from retrospective analyses of population-wide serological samples. Second, these samples

reveal the variety of exposures to different circulating strains and cross-reactivity beyond. Third, the serological samples may shed light on the asymptomatic infection rate when accompanied by clinical data. Finally, serological studies may provide information on vaccine coverage if the vaccine strains do not closely match the circulating strains, enabling the vaccine response to be differentiated from the infection. One of the major hopes of modern serological studies is to distinguish between a natural infection and immunization and to help modeling of future pandemics and influenza seasons. Modelers would notably benefit from data showing age-specific rates of asymptomatic infections by influenza virus type and subtype as well as data on cross-reactive immunity.

SEROPREVALENCE BEFORE AND AFTER THE 2009 INFLUENZA A(H1N1) PANDEMIC

By the end of March 2011, several serological studies of the immune response to the 2009 pandemic had been published (Table 2). Most of these studies demonstrated the existence of cross-reactive antibodies to the 2009 influenza A(H1N1) virus from earlier vaccinations or infections rather than exploring the epidemiology of the pandemic by means of serology.

Serology methods and limitations of the studies. Currently, the principal method for the laboratory diagnostics of influenza virus is reverse transcriptase PCR (RT-PCR) directly from a nasal or throat swab or a sputum specimen. Serological methods are mainly used in public health laboratories or specialized reference centers to determine the match between circulating strains and vaccine strains. For diagnostic purposes, paired acute- and convalescent-phase sera tested at the same time are the best samples. Traditional assays, such as the complement fixation test, measure the level of type-specific antibodies. The hemagglutination inhibition (HI) test can differentiate between the types and subtypes and is therefore more

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TABLE 1. Potential contribution of early timely seroprevalence data to mitigating influenza pandemics^a

Specific ECDC “known unknown” of pandemic	Rationale for knowing—the actions that may follow
Give estimates of susceptibility and then incidence and disease by age group or other risk parameters (e.g., those with chronic conditions and pregnant women)	Target interventions and refine countermeasures, e.g., who should receive antivirals and human avian influenza and specific pandemic vaccines
Determine key parameters for modeling and making estimations	Modeling of current and future cases, allowing rapid recasting of planning assumptions and resource deployment (“now-casting” and forecasting)
Broad estimate of severity of the pandemic, including age-related mortality and hospitalization rates for different influenza-related diagnoses	Determining the limits of public health actions that are justified

^a Adapted from reference 28a with permission of the publisher Elsevier.

widely used today. The virus neutralization and microneutralization (MN) tests assess the neutralizing antibodies against type, subtype, and strain of influenza virus. HI and MN tests determine the immunogenicity of vaccines as well as the level of antibodies resulting from natural infection and are the main methods used for influenza virus serology today. The variability of influenza virus serologic assays observed when comparing the results from different laboratories is mostly due to differences in protocols and endpoint analysis methods (37), but there are already published findings showing how the comparability of serologic assays can be improved by use of antibody standards (31), and projects have started to explore sharing of international standards for serology assays.

One of the limitations of the serology studies reviewed here is the heterogeneity in their populations and, in most of the early studies, the lack of pediatric population (Table 2). The comparability of the studies is mainly limited by differences in study populations and timing of sampling related to the pandemic. All published studies use slightly different reference sera and virus strains as well as cutoff values for positivity for the assay. Both HI and MN assays may also give cross-reactive results with other H1N1-subtype antibodies, resulting in overestimation of the prevalence of infection. The baseline seropositivity estimates are based on blood bank samples that may not represent the general population, usually excluding the pediatric population and mainly representing healthy adults. Many of the studies use serum bank samples as they are easily available and do not usually require an informed consent to be used. The limitations of these samples are the limited background data on the blood donors, selection bias introduced by including only healthy adults, the lack of pediatric samples, and minimal data on infections or vaccinations. Residual laboratory samples can include younger age groups but may also lack the background information and be biased toward hospitalized subjects with, e.g., a higher rate of underlying conditions. With serum bank and residual samples, it is also impossible to follow the development of the T-cell or other adaptive and innate immunity markers and changes, as well as the secretion of IgA antibodies. To better understand the full picture of influenza virus immunity, we would need to look at the different parts of the immune response in large cohort studies. Even smaller national serosurveys have become rare, and, e.g., in Europe, to our knowledge, fewer than five countries are regularly collect-

ing such samples and following the influenza virus seroprevalence.

Preexisting or cross-reactive antibodies against the influenza A(H1N1) 2009 virus. Some studies concentrated on the preexisting and cross-reactive antibodies that were present in the populations before the pandemic, suggesting some degree of immunity to the novel influenza virus (Table 2). The first report demonstrating cross-reactive antibodies was a U.S. study published approximately 2 months after the detection of the new virus (6). Using residual vaccine study sera of pediatric and adult cohorts, this study showed that cross-reactive antibodies were detected in 33% of adults aged over 60 years, that there were no cross-reactive antibodies in children, and that previous vaccinations did not protect against the novel influenza virus strain (6). A later study from the United States confirmed the early report from the CDC stating that 34% of persons born before 1950 had high antibody titers prior to the pandemic, as opposed to young adults and children, of whom only 4% had preexisting antibodies against the 2009 virus (15). In Finland, 56% of the participants over 90 years old had HI titers over 1:40 against the 2009 influenza H1N1 virus (19). Of the 70- to 79-year-olds, only 1.6% had such antibodies, and the younger age groups had none (19). In the United Kingdom, about 20% of the over-65-year-olds had cross-reactive antibodies against the 2009 virus before the pandemic while in the other age groups lower rates of seroprevalence were observed (16, 27). Similar patterns were found in Italy (30), Taiwan (18), New Zealand (5), and Australia (26), with over 60% of samples being positive in some cohorts older than 85 years (13). In contrast, in Singapore and Hong Kong, the baseline seroprevalence in all age groups was equal to or below 5% (9, 38), and in China no preexisting antibodies could be shown in rural farmers aged over 60 years (8). However, in Taiwan, 36% of nationwide serum samples from people over 75 years old had preexisting antibodies (18). In the same study, 0% of children under 5 years of age and less than 3% of younger adults (20 to 49 years old) had preexisting antibodies (18).

The antibodies against A(H1N1) 2009 showed cross-reactivity to the 1918 pandemic influenza virus. Several studies from different continents have shown high levels of cross-reactive antibodies to A(H1N1) 2009 in the serum of donors who had been exposed to the 1918 influenza virus (13, 19, 20), suggesting long-lasting immunologic memory of the initial influenza

TABLE 2. Summary of selected serosurveys published by March 2011^a

Study site (in alphabetical order)	Preexisting antibodies to A(H1N1) 2009 virus or cross-reactive antibodies to 1918 (H1N1) virus, % positive (age in yrs)	% seropositive after the pandemic (age in yrs)	Asymptomatic (%)	n	Comment(s)	Reference
Australia, blood donor samples	16 (16–24); 7 (25–34); 13 (35–44); 9 (45–54); 13 (55–64); 19 (>65)	37 (16–24); 22 (25–34); 15 (35–44); 16 (45–54); 20 (55–64); 25 (>65)		1,275		26
Australia, New South Wales, excess serum samples	0 (<5); 3 (6–12); 6 (13–18); 16 (19–35); 7 (36–65); 34 (66–85); 62 (>85); overall, 13 (0–>60)	16 (<5); 12 (6–12); 40 (13–18); 40 (19–35); 26 (36–65); 20 (66–85); 49 (>85); overall, 28		474 (before); 1,247 (after)		13
China, Guangxi province, rural farmers	0 (5–14); 3.1 (15–19); 3.6 (20–29); 5.6 (30–39); 4.2 (40–49); 1.7 (50–59); overall, 3.3	43 (5–14); 19 (15–19); 15 (20–29); 10 (30–39); 8.8 (40–49); 5.7 (50–59); overall, 14		4,043 (blood donors); 2,520 (outpatients); 917 (pediatric)	Rural only	8
China, Hong Kong, blood donor, hospital outpatient, and pediatric cohort samples	Done for a subset only	Done for a subset only	36	348		10
China, Hong Kong, index patient study, all ages			50	346	99% male, young adults	2
Finland, garrison cohort, after outbreak	1.6 (71–80); 2.1 (81–90); 5.5 (>90)	43 (30–39); 39 (40–49); 50 (50–59); 23 (>60)		1,031 (before); 225 (after)		19
Finland, stored serum samples	5 (1–4); 5 (5–9); 0 (10–14); 10 (15–19); 33 (20–39); 4 (40–59); 37 (>60)		45.2	587 (survey); 532 (HCW)		3
Germany, Frankfurt, stored serum samples	7 (<5); 12 (56–65); 22 (>65)	34 (1–4); 48 (5–19); 25 (20–39); 21 (40–59); 25 (>60)		1,147 (survey); 532 (HCW)		30
Italy, population-based serum samples	6.1 (1–4); 14 (5–19); 7.5 (20–39); 6.5 (40–59); 23 (>60)	53 (<2); 56 (3–9); 65 (10–19); 37 (20–29); 46 (30–49); 28 (50–64); 35 (65–79); 5 (>80); overall, 45		689 (before); 541 (after)		5
New Zealand, survey	0 (<2); 0 (3–9); 2 (10–19); 4 (20–29); 1.3 (30–49); 0 (50–64); 3 (65–79); 5 (>80); overall, 2	44 (15–19); 23 (20–24); 13 (25–29); 9 (30–39); 8 (40–49); 9 (50–59); 17 (>60)		50	Only adults	9
Norway, before, during, and after pandemic	5 (20–24); 5 (25–29); 1 (30–39); 3 (40–49); 2 (50–59)					32
Singapore, 4 adult cohorts, military, and community (in table)	0 (>51)			50	Only elderly, urban-based, small sample	7
Singapore, randomly recruited volunteers	20			295		18
Taiwan, hospital staff	0 (<5); 2.8 (20–49); 16 (50–74); 3.6 (>75); overall, 14	35 (0–4); 65 (5–14); 46 (15–24); 36 (25–44); 32 (45–64); 30 (>65)		1,255 (before); 2,225 (after pandemic, 2010)		16
Taiwan, vaccine study samples, nationwide	1.8 (0–4); 3.7 (5–14); 18 (15–24); 8.9 (25–44); 14 (45–64); 23 (>65)	39 (20–29); 33 (30–39); 30 (40–49); 34 (>50)		400		1
UK, residual microbiology and chemical pathology laboratory samples, eight regions of England	1.8 (0–4); 3.7 (5–14); 18 (15–24); 9.8 (25–49); 14 (50–64); 21 (65–74); 19 (75–79); 31 (>80)	23 (0–4); 46 (5–14); 38 (15–24); 15 (25–44); 12 (45–64); 24 (>65)		1,043		27
UK, Scotland, stored serum samples, after pandemic	2 (0–9); 5 (10–19); 13 (20–29); 10 (30–39); 14 (40–49); 11 (50–59); 13 (60–69); 48 (70–79); 59 (80–89); overall, 19	28 (0–9); 45 (10–19); 20 (20–29); 14 (30–39); 18 (40–49); 22 (50–59); 13 (60–69); 5 (70–79); 26 (80–89); overall, 21		846		40
UK, stored serum samples	4 (0–30); 34 (>60); boost effect with seasonal vaccination			107; 115 (boost effect)		15
USA, stored serum samples, all ages	89 (>55); 18% neutralizing against pdm virus, boost effect			116		25
USA, Tennessee, hospital staff	0 (children); 6–9 (18–64); 33 (>60)					6

^a The data are based on hemagglutination inhibition titers of >1:32 (in most studies, >1:40) or microneutralization assay titers of >160.

virus infection. The classic swine strain H1 and the contemporary human H1 sequences diverged from a common ancestor before 1918 (21). The persistence of such high-affinity antibodies might be due to similarities in the three-dimensional (3D) composition of the amino acids in the globular head of the hemagglutinin (HA) of the 1918 and 2009 pandemic influenza virus strains (11, 19, 39). These results could explain the reduced attack rates among older individuals during the 2009 pandemic. However, some studies, e.g., from countries with low seasonal vaccination rates or with different previous exposure to H1N1 influenza virus strains, have not demonstrated any cross-reactive antibodies in 40- to 80-year-olds (32). It was further shown that some of the cross-reactive antibodies against the pandemic H1N1 influenza virus are not neutralizing and would leave the subject susceptible to infection (25). Even in a population with a seroprevalence of 90%, only 18% had neutralizing antibodies (25). This is in line with the fact that A(H1N1) 2009 virus has been shown to induce low-avidity nonprotective antibodies and immune complex-mediated complement activation, which have been suggested to contribute to the severe and fatal cases (28).

The nucleotide sequences of nucleoprotein (NP) and matrix-1 (M1) protein of the A(H1N1) 2009 virus are more similar to those of the pandemic 1918 H1N1 strain than to those of seasonal H1N1 strains (14). However, Gras et al. have shown how the key epitope of NP has evolved from 1918 to 2009, being similar between 1918 and 2009 but displaying a different amino acid motif in the main positions from 1933 to 2006 (14). That study found only two main sets of core motifs that would be beneficial to include in vaccines (14) to induce generic immune responses to influenza viruses. The HA T-cell epitopes of seasonal and 2009 pandemic strains have also shown a significant level of conservation (12).

The reasons for the mild disease of the 2009 pandemic might be partly due to shared M1 protein epitopes of memory cytotoxic T lymphocytes (34); however, seasonal influenza virus vaccines did not induce cross-reactive antibodies against the 2009 strain in any age group (6, 15). The specific anti-influenza virus immunity has been suggested to last several decades, even a lifetime (19). Therefore, the host's immune response exerting pressure on antigenic drift appears to be the driving force of the evolution of influenza viruses.

Serial seroprevalence studies. Several serological studies have estimated attack rates, comparing samples from before and after the pandemic (Table 2). The first report was published by Miller et al. in March 2010 (27), presenting the results of United Kingdom samples taken in August and September 2009 after the first wave of the pandemic. These serum bank samples were compared with samples from 2008. Approximately one-third of 2008 samples from adults aged 80 years or older had cross-reactive antibodies to the 2009 pandemic influenza virus. Of the children, only 1.8% were positive (HI titer, >1:32) at that time. In the age group of over 25 years, no differences were seen between the 2008 and 2009 titers. However, in children there was a considerable increase in hemagglutination inhibition titers from 1.8% to 23% (0 to 4 years) and from 3.7% to 46% (5 to 14 years). This increase in titer was observed in London and the West Midlands, but not in more rural areas of the United Kingdom, suggesting an association of seroconversion with high-incidence areas (27). These results

were later complemented by a large study showing that the seroprevalence in school-aged children was as high as 65% (Table 2) after the second wave of the pandemic (16). In Scotland, approximately 40% of the adults were shown to have antibodies to the 2009 virus after the pandemic (1) (Table 2). The other published European studies of serial seroprevalence studies were carried out in Germany (3) and Norway (35). The results showed similar trends of antibody titers increasing mostly in younger age groups but also in the elderly (35).

In the United States, excess laboratory specimens representing all age cohorts born from the 1920s to 2010 were studied 2 to 4 weeks after the peak of the pandemic (November 2009) (40). An overall seroprevalence of 21% was found, which was greater than the baseline cross-reactivity of 6% to samples from 2008. In this study, the highest prevalence was among children aged 10 to 19 years (46%) and 0 to 9 years (28%). The only age group showing no increase in seroprevalence from 2008 was the 70- to 79-year-olds (5%). However, in the over-80-year-olds, seroprevalence and/or cross-reactivity was demonstrated for 26% of the samples (40).

Bandaranayake et al. published results of the seroprevalence in New Zealand (5). They had studied participants aged at least 1 year, recruited from general practices countrywide and from hospitals in the Auckland region. The pandemic samples were compared with prepandemic sera available from 2004 to early 2009. The overall pandemic seroprevalence was 27%. School-aged children had the highest prevalence, with an increase from 14% to 47% (5 to 19 years), followed by an increase in infants and young children from 6% to 30% (1 to 4 years). Adults aged over 60 years did not show any significant difference in seroprevalence in comparison of the cohorts of 2004 to early 2009 and 2009 after the pandemic. The study by Bandaranayake et al. showed differences in seroprevalence between ethnic groups (5). Pacific and Maori peoples had higher seroprevalences, as well as higher hospitalization and intensive care unit (ICU) admission rates, than did European-origin and other groups. However, no regional variation was observed, which is in line with an Australian study on blood donor samples covering five of the seven territories of Australia (26). In another Australian study, a significantly greater change in seroprevalence was observed after the pandemic in residents of Sydney than in other New South Wales residents (13).

Health care workers (HCW) did not show any significant difference in seroprevalence from the general population (5). In New Zealand, 18% of this population was found to have been exposed to the 2009 pandemic influenza A(H1N1) virus (5). This is higher than estimates derived from clinical surveillance data (4) but in line with an Australian study suggesting an overall infection rate of 16% (13). The population exposure suggested for Australia was 10% (26). Samples taken from different continents in the northern hemisphere from August to October 2009 showed variations in preexisting antibody proportions between countries but a similar overall trend toward higher proportions of antibodies in the elderly (33). In contrast to a study from Taiwan (7), the New Zealand study did not find any higher seroconversion rates in HCW. The study conducted on hospital staff and long-term care facility cohorts in Singapore (9) found that these cohorts had lower infection rates than did the general population. However, the vaccination

background of the different HCW cohorts is not known. Furthermore, in Singapore, only 13% of the community cohort seroconverted, compared with 26.7% in New Zealand.

A study in Singapore was conducted on four cohorts (general population, military personnel, staff from an acute-care hospital, and staff and residents from long-term care facilities) (9). Samples were collected before, during, and after the pandemic. This study and the United Kingdom's Fluwatch study are the only cohort studies published to date (22), having followed the same individuals before and through the pandemic. One limitation of the Singapore study was that no pediatric cohort was included, as the general population samples were from adults aged 21 to 75 years. However, some of the military personnel samples were from subjects aged 15 to 19 years. Chen et al. showed that younger age and working in the military were associated with higher infection rates (9). In the community and HCW, approximately 50% of the seroconverted participants remained asymptomatic (9). Overall, only 13% of the general population seroconverted, while in military personnel the rate was 29% (9). This is comparable with an investigation of a Finnish garrison outbreak, where 49% of the recruits were infected, of which 50% did not report any recent history of upper respiratory tract infection (2). Likewise, in the studies from Hong Kong and New Zealand, 36 and 45%, respectively, of seropositive individuals had no symptoms (5, 10).

In Hong Kong, patients from 14 outpatient clinics were recruited to a seroprevalence survey together with household members (10). This study reported the secondary attack rate and viral shedding of both A(H1N1) 2009 and A(H3N2) viruses to be similar (viral shedding of 5 to 7 days by RT-PCR and secondary attack rate of 8 to 9%) (10). In the patients for whom baseline and convalescent serology titers were available, no significant protection from an RT-PCR-confirmed infection could be shown (10). In another Hong Kong study, a large number of blood donor, hospital outpatient, and pediatric samples were studied (38) (Table 2). The overall attack rate was found to be 10.7% with almost 50% of the school-aged children being infected during the first wave of the pandemic (38). The older adults had higher risk of ICU admission and death in comparison with the infected children (38).

CONCLUSIONS AND LESSONS FOR FUTURE PANDEMICS

The best way to estimate infection rates for large outbreaks, such as the 2009 influenza A(H1N1) pandemic, at population level is to study serological cohorts. In such outbreaks, it is impractical to confirm most cases by laboratory diagnosis. Seroepidemiological studies allow us to refine estimates of the number of people at risk of infection, obtain infection fatality rate estimates, and inform policy on needs for vaccination and other countermeasures (Table 1). Equally, serological data make important contributions to early studies determining parameters required for refining planning assumptions. The currently published seroepidemiological studies of the 2009 influenza A(H1N1) pandemic have some findings in common, although there are many limitations in the data and in the processes of their timely determination.

Most studies looking into the seroprevalence prior to the pandemic found that cross-protective immunity from previous

infections or vaccinations increases with age, peaking in the >60-year-old age group (36). The studies with data from samples taken after the pandemic consistently found that the seroconversion rates were highest (20 to 60%) in children and teenagers (5, 27, 35, 36, 38, 40). The major differences in the results were in the studies from rural areas of China (8) and two studies from Singapore (9, 32), which showed very low levels of preexisting antibodies in the adult population in comparison to the other studies (Table 2). This has probably to do with the circulation of the viruses in previous seasons in these areas and within these populations as well as with the circulation of the A(H1N1) 2009 virus.

Like ordinary influenza seasons, the 2009 influenza A(H1N1) pandemic resulted in substantial proportions of both symptomatic and asymptomatic infections. A high proportion of asymptomatic infections has been demonstrated in studies from Finland, New Zealand, and Singapore that used a questionnaire to assess the symptoms of the blood donors during the study period. About half of the seroconverters reported no respiratory illness or febrile episode (2, 5, 9). In an index patient study in Hong Kong, more than a third of infected people did not have typical influenza-like illness symptoms (10). Taking together the results of the studies with pediatric cohorts (5, 10, 13, 16, 27, 35, 38, 40), approximately 30% of the children in various countries and different continents were infected. The adult populations, especially adults aged 60 years or more, seemed to have had cross-reactive antibodies from previous infections that protected them from illness. The early detection of these antibodies (6) and the knowledge that they did not protect from an infection with the 2009 virus were of crucial importance for the vaccination programs of the individual countries. The larger volume and distribution of the studies has provided us with a greater confidence in the results than looking only at the very few early studies. Overall, school-aged children played an especially important role in the transmission of influenza, and this was also shown in this 2009 pandemic (24). The pandemic 2009 virus has characteristics broadly similar to those of the seasonal H1N1 influenza viruses in terms of viral shedding, clinical illness, and transmissibility (10). Among the many differences between this pandemic and seasonal H1N1 epidemics were the preexisting protection in older people; the fact that the pandemic 2009 overall mortality among children was increased; the fact that many disease outcomes were unusually severe, especially in children, which placed hospitals under stress; and the fact that 30% of the deaths were among young healthy people (23). Additionally, the pandemic appeared outside the usual influenza season in the Northern Hemisphere (29).

The major findings of this review are that most likely 20 to 60% of children and teenagers were infected globally by the A(H1N1) 2009 virus and that 40 to 50% of the infections were asymptomatic. Many adults born before the 1950s had cross-reactive antibodies to the 2009 pandemic virus (13, 19, 20). However, only a minority of these antibodies were found to be neutralizing and hence protective of the elderly from their higher case-fatality (25).

In preparedness for the next pandemic or major epidemic, these seroprevalence studies support vaccination of specific population groups, such as children, ethnic groups, or people with underlying conditions who are at higher risk of disease

and severe outcomes. The results also justify vaccination of military personnel, as garrisons seem to favor virus transmission (2, 9). The vaccination of HCW and diagnostic laboratory personnel is important to minimize the risk for absenteeism during the periods of heavy workload. However, the performance and reporting of seroprevalence studies in the 2009 pandemic were suboptimal to achieve public health functions (Table 1). In the future, they would need to be prepared well in advance, so that they could be launched and conducted quickly at the time of the outbreak. Many laboratories that would have had the capacity for seroprevalence studies during the 2009 pandemic were hampered by the regulatory processes required for clinical studies and by the fact that staff were extremely busy undertaking other essential virological work. This should be overcome by preparation and preapproval of study plans for future pandemics, possibly taking advantage of preexisting mechanisms and protocols for other vaccine-preventable infectious diseases. Currently labor-intensive methods will benefit from the future implementation of high-throughput technologies and improvements in standardization. The better understanding of antigenic epitopes of influenza virus will contribute to the development of more-specific tests and help modelers to better predict immune protection based on antigenic properties of the circulating virus. The open sharing of data and analyses will also be fundamental for estimation of global burden of influenza.

There are several more seroepidemiological studies under way that should provide us with valuable information on the true attack rate of the pandemic virus, given that surveillance data tend to underestimate the infection rate. Annual influenza vaccine effectiveness and uptake data would allow estimation of the population protected from the infection. Standardization and improvement of laboratory methods to detect subtype-specific antibodies without cross-reactivity are crucial and will benefit the whole influenza laboratory network, not least by increasing comparability of results between laboratories worldwide. ECDC, WHO, and laboratories are taking measures to provide guidance and training as well as sharing of viruses and standards to achieve the best possible standardization of methods.

The immediate objectives of seroprevalence studies in a pandemic are to determine the likely preexisting immunity and to establish baselines and cohorts for detecting any change in seroprevalence during an epidemic or a pandemic (Table 1). The proportion of mild and asymptomatic infections as well as the likely proportions that remain susceptible needs to be investigated by seroprevalence studies after the early wave of a pandemic. After later waves, it is possible to determine the cumulative prevalence of infection in different age and risk groups, informing policy makers on the burden of disease and residual susceptibility. Together with the epidemiological and severity data, the seroprevalence studies may contribute to the decision making of targeting of vaccinations to specific groups. In summary, the applications of seroprevalence studies are many and the importance is high. Timeliness of the information is key, and therefore, seroprevalence studies should be supported at the national and supranational level and preparedness plans should be updated accordingly.

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Dr. Broberg has worked throughout her career in applied virology and diagnostics as well as committed herself to the improvement of diagnostic methods in the clinical diagnostic laboratory of the University of Turku. In 2010, she was appointed at the European Centre for Disease Prevention and Control as a virologist with a focus on influenza.

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Dr. Nicoll was dually trained in pediatrics and public health in the United Kingdom. Following clinical practice, he lived and worked in Africa from 1987 to 1991 on HIV and sexually transmitted infections (STI), establishing the Mwanza Programme. Then from 1991 to 2005, he worked with the UK Public Health Laboratory Service, which became the Health Protection Agency. He became head of its HIV and STD Division and then Director of the Communicable Disease Surveillance Centre from 2000 to 2005. During the severe acute respiratory syndrome (SARS) epidemic, he chaired WHO meetings reviewing epidemiology and control measures. He worked extensively in China as a visiting consultant for the World Bank on communicable disease control. From 2005 onward, as Influenza Coordinator at the ECDC from the UK Government and the HPA, he steered ECDC's extensive activities on influenza, including its response to avian influenza A(H5N1), its work on pandemic preparedness and seasonal influenza, and, with many others, its response to the 2009 A(H1N1) pandemic in 2009 to 2010.

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In 1993, he was appointed the first national Director of Public Health in the reformed Health Division in Malta, and in 2000, he was appointed Head of the Department of Public Health and Epidemiology of the University of Malta Medical School.

He has worked extensively in the international public health field, with agencies such as UNAIDS, WHO, the World Bank, UNICEF, and others, mainly on HIV/AIDS/STI issues. As Malta's first member of the new ECDC Management Board, he was present at the first meeting on 28 September 2004. In 2007, he joined the ECDC Surveillance Unit.