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# Estimating age-specific cumulative incidence for the 2009 influenza pandemic: a meta-analysis of A(H1N1)pdm09 serological studies from 19 countries

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**Background** The global impact of the 2009 influenza A(H1N1) pandemic (H1N1pdm) is not well understood.

**Objectives** We estimate overall and age-specific prevalence of cross-reactive antibodies to H1N1pdm virus and rates of H1N1pdm infection during the first year of the pandemic using data from published and unpublished H1N1pdm seroepidemiological studies.

**Methods** Primary aggregate H1N1pdm serologic data from each study were stratified in standardized age groups and evaluated based on when sera were collected in relation to national or subnational peak H1N1pdm activity. Seropositivity was assessed using well-described and standardized hemagglutination inhibition (HI titers  $\geq 32$  or  $\geq 40$ ) and microneutralization (MN  $\geq 40$ ) laboratory assays. The prevalence of cross-reactive antibodies to the H1N1pdm virus was estimated for studies using sera collected prior to the start of the pandemic (between 2004 and April 2009); H1N1pdm cumulative incidence was estimated for studies in which collected both pre- and post-pandemic sera; and H1N1pdm seropositivity was calculated from studies with post-pandemic sera only (collected between December 2009–June 2010).

**Results** Data from 27 published/unpublished studies from 19 countries/administrative regions – Australia, Canada, China, Finland, France, Germany, Hong Kong SAR, India, Iran, Italy, Japan, Netherlands, New Zealand, Norway, Reunion Island, Singapore, United Kingdom, United States, and Vietnam – were eligible for inclusion. The overall age-standardized pre-pandemic prevalence of cross-reactive antibodies was 5% (95%CI 3–7%) and varied significantly by age with the highest rates among persons  $\geq 65$  years old (14% 95%CI 8–24%). Overall age-standardized H1N1pdm cumulative incidence was 24% (95%CI 20–27%) and varied significantly by age with the highest in children 5–19 (47% 95%CI 39–55%) and 0–4 years old (36% 95%CI 30–43%).

**Conclusions** Our results offer unique insight into the global impact of the H1N1 pandemic and highlight the need for standardization of seroepidemiological studies and for their inclusion in pre-pandemic preparedness plans. Our results taken together with recent global pandemic respiratory-associated mortality estimates suggest that the case fatality ratio of the pandemic virus was approximately 0.02%.

**Keywords** A(H1N1)pdm09, cross-reactive antibodies, cumulative incidence, H1N1pdm, seroprevalence.

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## Introduction

Soon after detection of the novel pandemic influenza A (H1N1)2009 virus (H1N1pdm) in Mexico and the United States in April 2009,<sup>3</sup> countries across the globe began reporting laboratory confirmed H1N1pdm cases to the World Health Organization (WHO).<sup>4</sup> However, as case numbers increased, laboratories were overwhelmed with demand for testing. WHO responded with new guidance in June 2009 asking that countries report the first cases detected in a country, that testing focus on fatal and severe cases, and for countries to only report fatal cases to WHO.<sup>5</sup> As a result, by the time, the pandemic was declared over in August 2010,<sup>6</sup> numbers of cases and deaths (<1 million and >18 449,<sup>7</sup> respectively) reported to WHO represented only a small fraction of the true burden of infection and mortality due to H1N1pdm.

Even in well-resourced countries, the very large numbers of H1N1pdm cases, the non-specificity of clinical case definitions for influenza, and finite testing capacity means that incidence cannot be estimated from case-based surveillance. This information is critical to understanding the overall morbidity, mortality, and population-level severity of the H1N1pdm virus, as it serves as the denominator for the estimation of severity measures. Along with population-level surveillance to capture numerators (i.e., H1N1pdm, hospitalizations and deaths), representative serological studies are designed to collect denominator data (i.e., infections) that can be used to estimate severity parameters such as the CFR (i.e., the total number of H1N1pdm deaths divided by the total number of H1N1pdm infections) and hospitalization ratios (number of H1N1pdm hospitalizations divided by H1N1pdm infections). Thus, analysis of serological data can provide accurate measures of incidence, reduce the uncertainty around severity assessment, and help inform the appropriate intensity and targeting of mitigation policies.<sup>8–10</sup>

As well as estimating the proportion of the population infected by a particular virus, data from seroepidemiological studies can provide insights into age-specific and regional trends in incidence and cross-protective immunity, which are important to characterize the infectivity of a new virus, identify key target groups for interventions and for developing mitigation measures.<sup>8–11</sup> Insight into cross-protective (or partial) immunity acquired from exposure to other influenza strains or vaccination is of particular scientific interest. Knowing what proportion of the population had antibodies before the first wave and how this immunity affected subsequent circulation of the virus provides valuable information for understanding the transmission dynamics of influenza pandemics more generally.

A number of early seroepidemiological studies using residual sera collected prior to the start of the H1N1pdm

pandemic were conducted within months of identification of the H1N1pdm virus to assess the level of pre-existing immunity in the population by age, quickly followed by investigations from a number of countries to estimate the proportion of the population infected with the H1N1pdm virus.<sup>12</sup> Together with early investigations elucidating age-specific clinical attack rates<sup>13,14</sup> and transmission characteristics<sup>15</sup> of the new virus, these studies provided critical input into and reduced uncertainty around national and global policy decisions. Numerous seroepidemiological studies have subsequently been published, but the comparison and direct interpretation of the results of serological studies is difficult due to the varied epidemiological methods used to collect sera, the heterogeneity in the populations under study, variation in laboratory assays used, and criteria for seropositivity.<sup>12,16</sup>

The objective of this study is to bring together all available original serological data in a standardized format from H1N1pdm seroepidemiological studies to estimate the proportion of the population with cross-reactivity antibodies to H1N1pdm prior to the start of the pandemic and to estimate age-specific cumulative incidence of H1N1pdm infection during the first year of the pandemic. This study builds upon the findings of Kelly *et al.*<sup>17</sup> by including a number of additional H1N1pdm serologic studies conducted from a number of additional countries since this publication. Combined with what is known about morbidity and mortality of the pandemic virus around the world, these estimates provide a better sense of the overall global impact of the H1N1 pandemic.

This study represents the combined work and collaboration of influenza researchers from more than 27 different research groups around the world and is the first of its kind to use original data to produce a summary estimate from a global perspective of the proportion of the population that was infected during the first year of the influenza pandemic of 2009. Our analysis includes original serologic data from several low- and middle-income countries including China, India, Iran, Vietnam, and Reunion Island and high-income countries, including Australia, Canada, Finland, France, Germany, Hong Kong SAR, Italy, Japan, Netherlands, New Zealand, Norway, Singapore, United Kingdom, and the United States.

This work provides critical insight into the underappreciated impact and severity of the pandemic, and our results are of great value in planning and preparing for the next pandemic. Age-specific cumulative incidence rates are critical parameters used by public health decision makers and mathematical modelers in planning for and responding to a pandemic and provide accurate denominator estimates to calculate a key parameter – the case fatality ratio. Together with recent<sup>1</sup> and forthcoming<sup>2</sup> estimates of H1N1pdm mortality – the numerator of the case fatality ratio (CFR) –

and our summary cumulative incidence results, we suggest that the CFR of the pandemic virus was approximately 0.02% providing insight into the severity of the 2009 influenza pandemic globally.

## Methods

An extensive literature search for H1N1pdm serological studies was conducted using a keyword-based computerized search of the National Library of Medicine through PubMed. The search was limited to all H1N1pdm seroepidemiological studies published by 1 January 2012. Articles with the MeSH keywords: human influenza, pandemic, sero-incidence, and seroprevalence, in their titles or abstract were reviewed for eligibility for inclusion. The references cited in screened articles were further inspected by SH and MDVK to identify additional relevant studies (any discrepancies were discussed with AWM; Figure 1a). In addition to published studies, the WHO Global Influenza Programme contacted researchers known to be conducting serological studies from a comprehensive list of planned and ongoing H1N1pdm serological studies compiled and maintained by WHO. Additionally, a further effort was made to identify unpublished studies by contacting experts and known influenza researchers by searching influenza conference proceedings and country surveillance agency reports. Researchers of unpublished studies were asked to share their study methodology (further details below) and preliminary results to allow assessment for inclusion. As with published studies, unpublished data were also used only if data were available by 1 January 2012.

### Inclusion/exclusion criteria

#### *Inclusion criteria*

Published and unpublished studies that measured overall and age-stratified antibody titers against H1N1pdm 2009 influenza virus by well-described and standardized hemagglutination inhibition (HI) and microneutralization (MN) laboratory assays were included. Briefly, seropositivity was assessed as assay HI titers  $\geq 32$  or MN assay  $\geq 40$ . Additionally, serological studies that measured cross-reactive antibodies to H1N1pdm influenza virus in sera collected prior to the start of the 2009 pandemic were included to quantify age-stratified pre-existing cross-reactive antibody levels in populations. To be included, authors of individual studies were required to provide results in harmonized age groups (0–4; 5–19; 20–44; 45–64 and  $\geq 65$  years old) and additional details about their study population (e.g., specific start and end dates for sera collection, sample size in each age group, assay and criteria for seropositivity, description(s) of study populations from

which sera was used, specific location(s) of residence of subjects providing sera, and use of seasonal and pandemic vaccination among included sera, if possible). When use of H1N1pdm vaccine was available in individual studies, we asked authors to provide results among unvaccinated persons only.

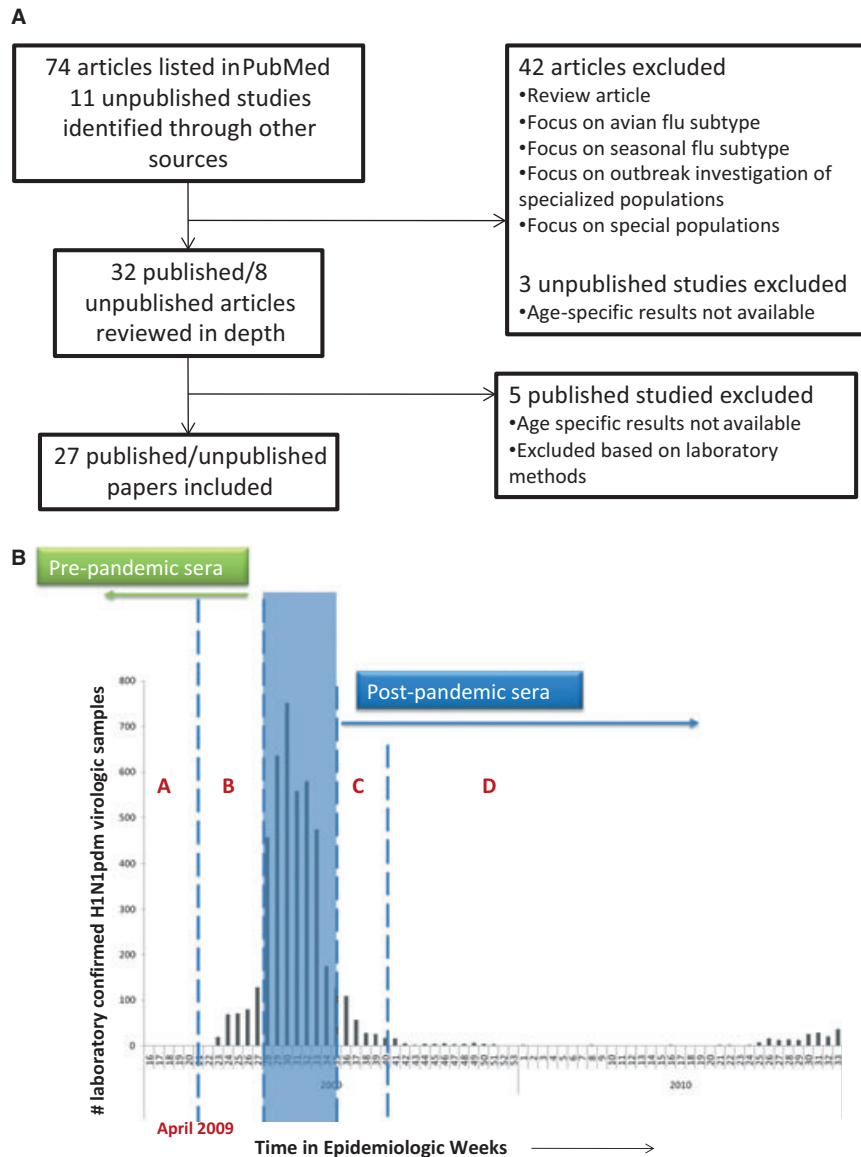
#### *Exclusion criteria*

Clinical vaccine trials were excluded, as were serological studies of avian and seasonal (H1N1 or H3N2) influenzas. Additionally, studies of populations in closed settings (i.e., military facilities, schools) or among specific populations only (e.g., HIV-infected individuals or pregnant women) were excluded. Finally, studies that included only H1N1pdm vaccinated individuals were excluded.

### Data abstraction, synthesis and statistical methods used for metaanalysis

Data from included studies were categorized based on when sera were collected in relation to national, or subnational where available, 2009–2010 virologic H1N1pdm activity<sup>18</sup> (Figure 1b; categories: pre-pandemic sera, pre- and post-pandemic sera and post-pandemic sera only). Studies that only collected sera during the peak of H1N1pdm virologic activity were excluded from the analyses (Figure 1b, shaded area). For all three different sets of analyses: overall and age-specific prevalence of cross-reactive antibodies to the H1N1pdm virus using pre-pandemic sera, overall and age-specific cumulative incidence using studies with both pre- and post-pandemic sera and overall and age-specific seroprevalence using studies with post-pandemic sera only, we used random effects (at the study level) logistic regressions to obtain pooled overall and age-specific estimates as well as to take into account the heterogeneity of results between studies.

A database was created (by SH and MDVK) to collate extracted information from each study including: country of study, author and year of publication, laboratory assay(s) used, cut-off value used for determining seropositivity, description of study population from whom sera was collected, period(s) when sera were collected, sample size, proportion seropositive with 95% confidence intervals, timing of the national peak pandemic activity for the relevant country according to data reported to FluNet,<sup>18</sup> timing of H1N1pdm vaccination campaign for the country, use of seasonal vaccine among study population (if available), and difference between timing of sera collection and H1N1pdm peak virologic activity (in weeks). Because different studies used different age categories for reporting seropositivity results in their individual publications, we requested all researchers to share their seropositivity results for five age categories (0–4; 5–19; 20–44; 45–64, and  $\geq 65$  years old) to ensure comparability. These age



**Figure 1.** (a) Review process of published and unpublished H1N1pdm serologic literature search. (b) Example of the characterization of timing of sera collection in relation to national H1N1pdm virus activity. N.B. Characterization of sera timing was conducted using the national, or subnational when available, epidemic curve separately for each country that provided serological data. Time period A indicates the time period prior to the reporting of the first H1N1pdm cases in North America and start of the 2009 influenza pandemic. Time period B indicates the time period after the H1N1pdm virus was identified in North America, but before wide-spread circulation of the virus occurred in each country. This assessment was made for each individual country or subnational geographic area if subnational virologic data were available. Time period C indicated the time after the national or subnational peak in H1N1pdm virologic activity was over, but not completely back to baseline levels. Time period D indicates the national or subnational time when H1N1pdm virus circulation was clearly over. Shaded area indicates example of peak H1N1pdm virologic activity. Studies that collected sera during peak activity were excluded from the analyses.

categories were chosen based on differences in the epidemiology and reported clinical severity of the disease in these age groups.<sup>19</sup> Overall pooled estimates were age-adjusted using age-specific population estimates from the UN.<sup>20</sup> To evaluate seroprevalence levels over time, we explored age-specific post-pandemic seroprevalence versus the difference in timing of sera collection and the national peak of H1N1pdm virus activity.

### Pre-pandemic sera to estimate prevalence of cross-reactive antibodies to the H1N1pdm virus

All sera collected prior to April 1 2009, regardless of study design, were classified as pre-pandemic sera for which baseline overall and age-specific cross-reactive antibodies to the H1N1pdm virus were estimated (Figure 1b). We modeled overall and age-specific pre-pandemic prevalence of cross-reactive antibodies from studies with sera collected



prior to April 2009 (Figure 1b, area indicated as time period A) and studies that included sera collected prior to widespread community transmission (see Figure 1b, time period B). We then explored, in addition to other possible causes of heterogeneity (described below), whether study timing explained any of differences (i.e., whether the pre-pandemic prevalence of cross-reactive antibodies differed between studies conducted at time period A versus B). Only studies that analyzed seropositivity using HI were included in pre-pandemic analyses. Details of the included studies are provided in Table S1.

### Pre- and post- pandemic sera to estimate cumulative incidence

For studies that had both pre- (Figure 1b time period A or B) and post- pandemic sera (Figure 1b, time period C or D) according to the national or subnational period of H1N1pdm virus circulation, overall and age-specific cumulative incidence were calculated for each study by taking the difference in seroprevalence. In included studies, sera were collected twice from the same subject (paired sera from longitudinal studies) or twice in the same population but from different individuals (unpaired sera from cross-sectional studies) before the start of the pandemic and after the pandemic was over. Studies that analyzed seropositivity by HI and MN were included in incidence calculations. Details of the included studies are provided in Table S2.

### Post-pandemic sera to estimate H1N1pdm seroprevalence

Finally, we modeled and provided pooled overall and age-specific H1N1pdm seroprevalence from post-pandemic sera, that is, sera collected during time periods, which coincided with a decline in national or subnational H1N1pdm transmission (Figure 1b, time period C) or when transmission ceased (Figure 1b, time period D). Only studies that analyzed seropositivity using HI were included in post-pandemic analyses. Details of the included studies are provided in Table S3.

### Meta-regression

We explored differences in the outcomes listed above for all three sets of analyses, by adjusting for one covariate at a time in the random effects logistic regressions. Such models allow for within and between study variation to be included in the estimated coefficients. The covariates considered in the relevant univariable random effects logistic regressions were: study timing for the pre- and post- pandemic single sera analyses (i.e., we examined whether there were differences: (i) for the pre-pandemic studies, between studies conducted at time period A and B in Figure 1b, and (ii) for the post-pandemic single sera studies, between studies conducted at time period C and D in Figure 1b, respectively); assay

(HI  $\geq$  1:32; HI  $\geq$  1:40; MN  $\geq$  1:40; for estimates of H1N1pdm cumulative incidence, only); subject type; country and geographic region of sera collection; if H1N1pdm vaccination was used in the included countries; and population density at the national level.<sup>21</sup>

## Results

### Included studies

Seventy-four articles were identified for title and abstract review, and 32 full-text articles were retrieved and reviewed (Figure 1a). Twenty-seven studies, including eight unpublished studies (at the time of data collection), were included in the meta-analysis (Table 1). Of those, 19 studies from 15 countries included pre-pandemic sera in which overall and age-specific prevalence of cross-reactive antibodies were estimated<sup>22–40</sup> (details of included studies are shown in Table S1); 12 studies from 11 countries contained both pre-and post-pandemic in which overall and age-specific H1N1pdm cumulative incidence were estimated<sup>22,23,29–34,38,39,41–43</sup> (Table S2); and 10 studies from nine countries contained post-pandemic sera in which overall and age-specific H1N1pdm seroprevalence were estimated<sup>35–37,44–49</sup> (Sridhar S, personal communication; Table S3).

In total, our analysis was based on approximately 90 000 serological samples from 19 countries and/or administrative regions, including Australia, Canada, China, Finland, France, Germany, Hong Kong SAR, India, Iran, Italy, Japan, Netherlands, New Zealand, Norway, Reunion Island, Singapore, the United Kingdom (UK), the United States (US), and Vietnam (Figure 2). Pre-pandemic seroprevalence data were available from Chinese Taipei, but excluded from the pooled results because results were only available by MN (Sridhar S, *et al.* personal communication, Chen M. personal communication).<sup>9,22–28,30–32,34–39,41,42,44,46–51</sup> Pre- and post-pandemic sera from Greece were excluded from the cumulative incidence results because seropositivity was analyzed by enzyme-linked immunosorbent assay (ELISA), a novel method developed by the researchers and not fully validated.<sup>52</sup>

### Pre-pandemic prevalence of cross-reactive antibodies

The pre-pandemic prevalence of cross-reactive antibodies were estimated from pooling serological data from 15 countries from 19 studies (n sera = 15 476). The overall age-adjusted pre-pandemic prevalence of elevated cross-reactive H1N1pdm antibodies was 5% (95%CI 3–7%; Table 1; Figure 3A). Prevalence increased with age (Figure 4A; 0–4 years old 1% [0.3–4%], 5–19 years old 4% [1–9%], 20–44 years old 5% [3–8%], 45–64 years old 5% [2–9%]) and was highest in subjects 65 years and older (14%

**Table 1.** Characteristics of included studies for each of the age-specific and age-standardized pooled estimates

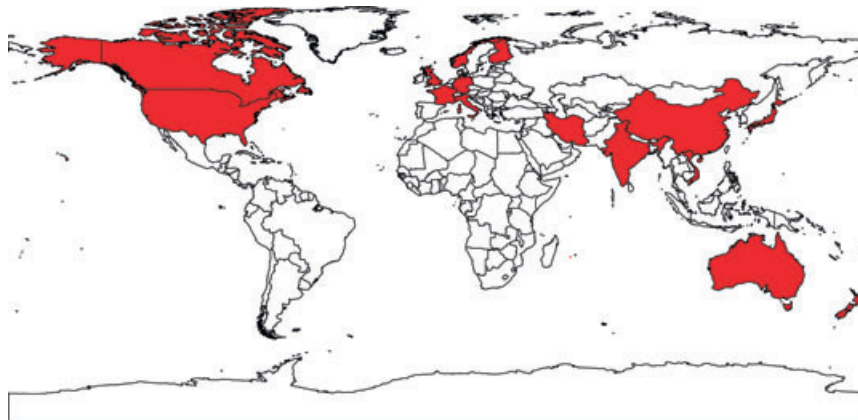
| Estimate  | Age-specific H1N1pdm cross-reactive antibodies  | Age-specific H1N1pdm cumulative incidence  | Age-specific H1N1pdm seroprevalence   |
|---|---|--|---|
| Description of sera included in estimate          | Studies, which included pre-pandemic sera   | Studies, which included both pre- and post-pandemic sera   | Studies, which included post-pandemic sera (only)   |
| Source of sera ( <i>n</i> countries)              | Australia, Canada, China, Finland, France, Germany, India, Italy, Japan, New Zealand, Norway, Reunion Island, Singapore, UK, USA (15) | Australia, Canada, France, Germany, Hong Kong SAR, Japan, New Zealand, Norway, UK, USA, Vietnam (11) | Canada, China, France, Germany, Iran, Netherlands, Reunion Island, Singapore, UK, USA (9) |
| Number of studies included in estimates           | 19  | 12   | 10  |
| Number of sera samples included in analyses       | 15 476  | Pre-pandemic sera = 9910<br>Post-pandemic sera = 14 228  | 52 479  |
| Assays used and criteria for seropositivity       | HI $\geq$ 1:32* or HI $\geq$ 1:40   | HI $\geq$ 1:32; HI $\geq$ 1:40; MN $\geq$ 1:40   | HI $\geq$ 1:40**  |
| Overall age-standardized pooled estimate (95% CI) | 5% (3–7%)   | 24% (20–27%)   | 32% (26–39%)  |

See Tables S1–S3 in the Supporting information for details of individual studies.

HI, hemagglutination inhibition; MN, microneutralization assay.

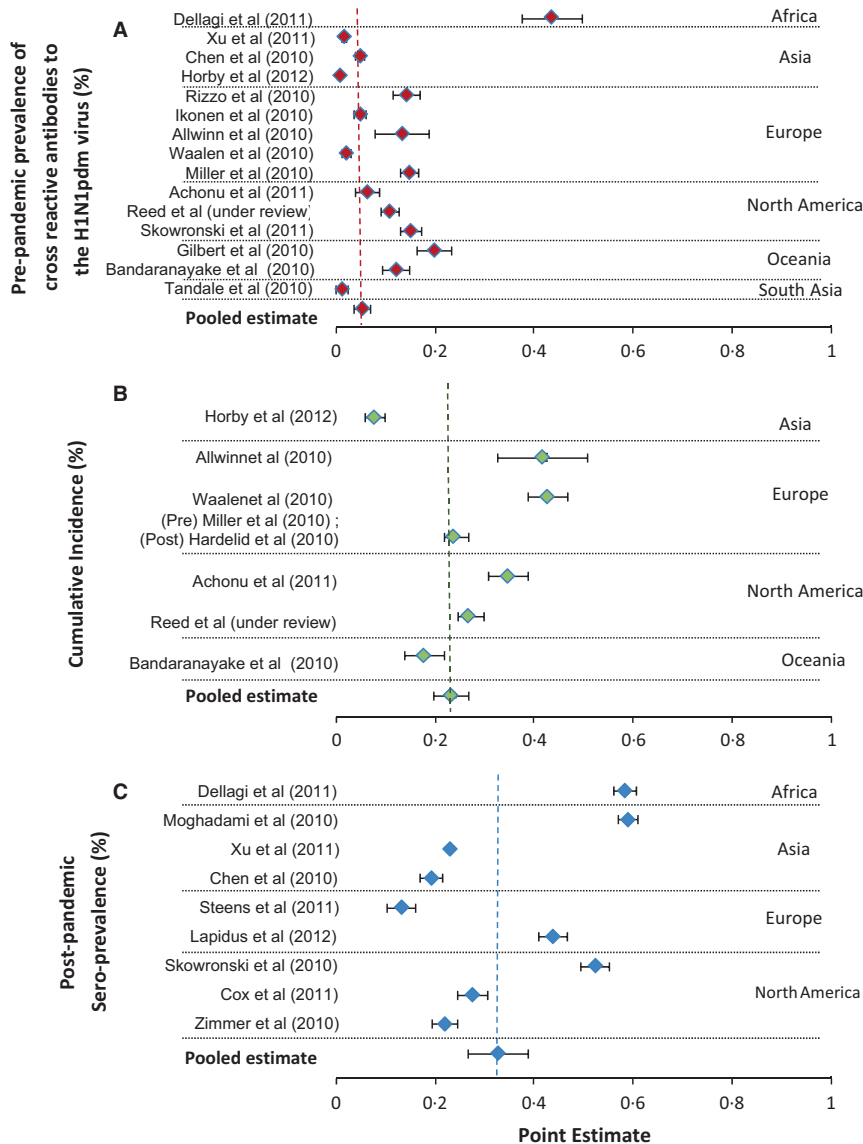
\*Hardelid *et al.*<sup>41</sup> and Iwatsuki-Horimoto *et al.* (2011) only; all other studies used HI  $\geq$  1:40 as criteria for seropositivity.

\*\*All studies in H1N1pdm seroprevalence estimates used HI  $\geq$  1:40 as criteria for seropositivity.

**Figure 2.** Geographic distribution of included study populations.

[8–24%]). Overall, there were significant differences in prevalence by region, with individuals from Asia less likely and subjects from one site in Africa (Reunion Island) more likely to have cross-reactive antibodies to H1N1pdm when compared with Europe (OR = 0.098 95%CI 0.01–0.9; OR = 9.2 95%CI 1.9–43.8), respectively). Subjects from one site in Africa also had higher seroprevalence among 5–19 (OR = 14.2 95%CI 1.2–174.9), 20–44 (OR = 6.9 95%

CI 3.3–14.4), 45–64 (OR = 21.4 95%CI 4.2–110.0) and  $\geq$  65 (OR = 17.0 95%CI 2.3–127.2)-year-old age groups when compared with individuals from Europe in the same age groups. Subjects 20–44 years old from Asia had lower seroprevalence when compared with Europe (OR = 0.20 95%CI 0.1–0.4). Subjects from one study of rural households (Vietnam) had lower overall pre-pandemic seroprevalence than outpatients (OR = 0.06 95%CI 0.004–0.8). There were



**Figure 3.** Cumulative incidence of H1N1pdm infection in studies with pre- and post-pandemic sera collection. Each dot represents the unadjusted point estimate with 95% confidence bounds for a study that provided data from all age groups. Individual study estimates are unadjusted; the pooled estimate is age-adjusted. There was heterogeneity in the overall rates variance estimates for the random effects for the pre-pandemic overall estimates (A)  $P = 0.024$  and post-pandemic seroprevalence rates (C)  $P = 0.09$ ; and the  $I^2$  for the overall cumulative incidence rates (B) =98.1%.

no significant differences in pre-pandemic seroprevalence and any other covariate under investigation.

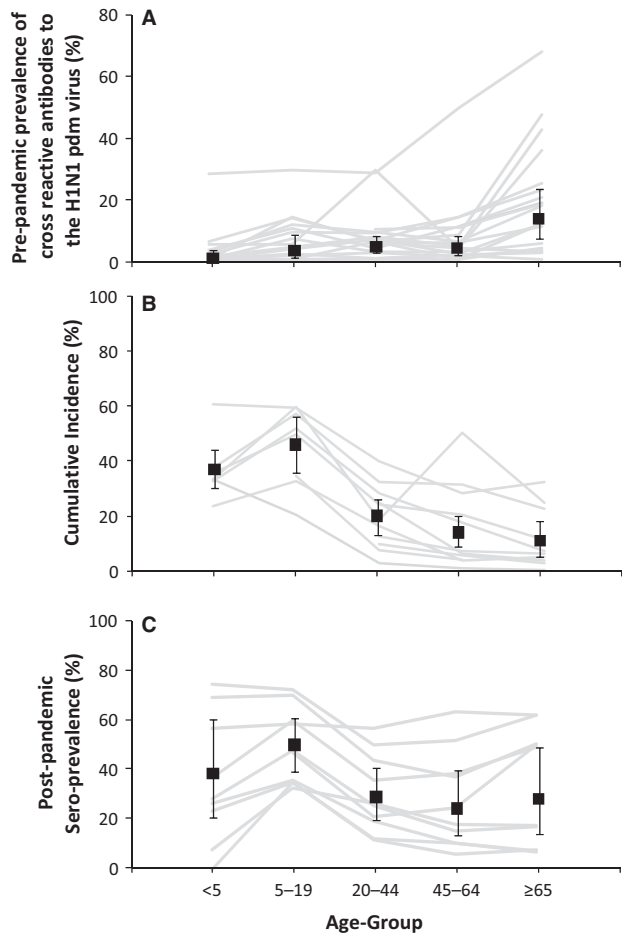
### Cumulative incidence of pandemic influenza infection

Data used to estimate age-specific cumulative incidence were available from 11 countries and 12 studies (Table 1; Table S2). The overall age-adjusted cumulative incidence of H1N1pdm infection based on the difference between pre- and post-pandemic seroprevalence was 24% (95%CI 20–27%, Figure 3B) and varied significantly by age (Figure 4B).

The highest age-specific incidence was found among children 5–19 years old (46% [36–56%]), followed by 0–4 years old (37% [30–44%]) and decreased by age from 20 years old and older (20–44 years old 20% [13–26%], 45–64 years old 14% [9–20%]). The lowest incidence was found in those  $\geq 65$  years old (11% [5–18%]).

There were significant associations found between incidence and region and subject type in the overall estimate, indicating that overall cumulative incidence was 28% lower (95%CI 7.7–48.4) in Asia when compared with Europe and 23% lower (95%CI 3.1–42.7) in subjects from rural





**Figure 4.** Age-specific (A) prevalence of cross-reactive antibodies from baseline pre-pandemic sera, (B) cumulative incidence of H1N1pdm infection using pre- and post-pandemic sera and (C) H1N1pdm seroprevalence from post-pandemic sera. Point estimates indicate pooled estimate and lines represent relevant 95%CI. Each line represents unadjusted age-specific results from individual studies. See Tables S1–S3 for studies included in each estimate.

households (Vietnam) compared with countries sampling from outpatients. Samples from subjects 5–19 years old from Asia and Oceania had lower cumulative incidence than samples from Europe in the same age group (29% [95%CI 15.8–41.9] lower, 21% [95%CI 3.1–39.3] lower, respectively). Countries that may have included persons between the ages of 5–19 and 20–44 vaccinated with the pandemic vaccine in their sampled population had higher cumulative incidence than countries that excluded H1N1pdm vaccinated persons in these same age groups (19% [95%CI 3.4–33.8] higher, 15% [95%CI 3.1–26.1] higher, respectively). When we exclude the two countries, which suggested that a significant proportion of their study populations' seroprevalence may be due to vaccination (Norway<sup>34</sup> and the US<sup>39</sup>), rather than natural infection, the overall pooled cumulative incidence is 21% (95%CI 18–25; see Figure S1) compared with 24% (95%CI 20–27%).

### Post-pandemic seroprevalence

Post-pandemic seroprevalence was estimated by pooling data from nine countries from 10 studies ( $n$  sera = 52,479; Table 1). The overall age-adjusted H1N1pdm seroprevalence was 32% (95%CI 26–39%; Figure 3C). From age 5, seroprevalence generally, although not significantly, decreased with age (Figure 4C) and decreased, not significantly, across all groups with increasing time interval between sera collection and peak in influenza virus activity (data not shown). There were no significant associations between overall seroprevalence and any covariate examined. However, for the 0–4 year old age group, a lower proportion sampled after the epidemic wave was over (Figure 1b, time period D) were seropositive compared with sera collected during the decline of the epidemic (Figure 1b, time period C; OR = 0.16 95%CI 0.04–0.6). In addition, for the 0–4 year old age group, countries that may have included persons vaccinated with the pandemic vaccine in the sampled population had lower seroprevalence than countries that excluded H1N1pdm-vaccinated persons (OR = 0.21 95%CI 0.06–0.8).

### Discussion

Our study is the first to gather and analyze primary H1N1pdm serologic data in standardized age groups from countries/administrative regions across the world. Our results suggest that approximately 20–27% of the populations in the included countries were infected with H1N1pdm virus during the first year of circulation. Incidence was highest in the 5–19 years age group, where approximately 46% (95% CI 36–56%) were infected, and lowest in the  $\geq 65$  age group, where approximately 11% (95% CI 5–18%) were infected. Although, as expected, there was some local within-country variation in infection rates as demonstrated by individual studies, we found consistency in age-specific cumulative incidence estimates across countries. This consistency in estimated infection rates by age group between countries may have been strengthened in part because we consistently categorized our sera based on timing of collection in relation to peak H1N1pdm viral activity in each country. Assuming that the cumulative incidence in the countries included in our studies is similar to the rest of the world for which no little data exist and if the global mortality estimates produced by two research groups<sup>1,2</sup> are confirmed by other studies, this would place the CFR for H1N1pdm at <0.02%.

Our results are consistent with our estimates of H1N1pdm seroprevalence using post-pandemic sera and with other H1N1pdm seroprevalence studies recently or not yet published from Iceland, Mexico, Chinese Taipei, India, Mongolia, Mali,<sup>53–56</sup> Laos, Djibouti, and Bolivia (CoPanFlu-International consortium unpublished data, personal communication from X. de Lamballerie), with a study from

Greece with pre- and post-pandemic sera that was excluded from our analyses<sup>51</sup> but are slightly higher than the overall estimated cumulative incidence found in one analysis.<sup>17</sup> This may be because our study includes a number of additional middle and low countries who conducted serologic studies because this analysis was published and because we excluded studies which focused on specialized populations<sup>17</sup>). Additionally, the age-specific trends we found in our cumulative incidence results are consistent with studies which measured cumulative incidence as a fourfold increase in titers among paired sera<sup>36,42,57</sup> and similar to studies which measured age-specific secondary attack rates using RT-PCR.<sup>58,59</sup>

In the analyses of pre-pandemic data, we found increasing levels of cross-reactive antibodies to H1N1pdm virus with age, although there were differences in these patterns by region. For example, older individuals in some Asian countries had lower levels of cross-reactive antibodies prior to widespread circulation of the pandemic strain than did individuals in other regions. However, this was not a universal finding for all Asian countries and may be a reflection of the age groups we chose in this meta-analysis because we collapsed elderly age categories into a single unit ( $\geq 65$  years of age): some studies observed differences among the elderly ( $>65$  years old) versus very senior individuals (i.e.,  $>80$  years old, e.g.,<sup>25,41,52,60</sup>). We note that regional differences did not persist when only looking at cumulative incidence from studies in which two sets of sera from one population were tested in the same laboratory using the same methodology or in post-pandemic seroprevalence. Therefore, given the small numbers of studies in individual regions, these patterns may reflect differences in laboratory methodology. However, this does not rule out the possibility that some serologic assays fail to identify antibodies in older individuals, or reflect antibodies among older individuals in countries without high routine seasonal influenza vaccination coverage.<sup>61</sup> Differences in laboratory methodology rather than real differences in pre-existing immunity would also explain the observation that reported cumulative incidence was not higher in  $\geq 65$  year olds in Asia where pre-pandemic seroprevalence was found to be lower.<sup>62</sup> We also observed low-level pre-pandemic seropositivity in children ( $<5\%$ ) and adolescents ( $<10\%$ ) in some countries, which may again be due to assay differences between laboratories.

There are a few factors that may affect the accuracy of our estimates. The inherent limitations of combining results from influenza serologic studies have been widely discussed<sup>12,16,63</sup> and could have an impact on the accuracy of our estimates. Based on our analyses, we strongly support the recommendations to standardize influenza seroepidemiological studies both in terms of epidemiologic and laboratory methods. In addition, declining antibody levels over time in some of the populations studied and the fact that not all

laboratory confirmed H1N1pdm patients seroconvert<sup>37,64–67</sup> could have resulted in our results slightly underestimating the true incidence. We found limited evidence of a decline in the proportion seropositive over time when looking at the timing of post-pandemic sera collection in relation to the peak in H1N1pdm virus activity (data not shown). We found conflicting results with respect to the impact of vaccination in our cumulative incidence estimates and post-pandemic estimates. Because of this and because vaccine coverage in most of the included countries had reached little of the population at the time sera were collected (e.g., in the United States<sup>39</sup>), and the observed increase was not in age groups targeted for vaccination, we believe that the H1N1pdm vaccination has had little impact on our overall cumulative infection and seroprevalence estimates results. When we excluded studies that suggest that seroprevalence may be due to vaccination,<sup>34,39</sup> rather than natural infection, the overall pooled cumulative incidence reduced slightly.

Finally, we were unable to include serological data in our pooled estimates from all regions of the world – notably from mainland Africa and Latin America, where to our knowledge, no H1N1pdm09 seroprevalence data exist. Despite this, however, we believe that H1N1pdm incidence may have been similar in all parts of the world because reported mortality rates and published reports of influenza activity in Latin America and Africa were similar to those reported in Europe and North America,<sup>54,68,69</sup> and to those reported in the countries included in our study. The lack of H1N1pdm morbidity, mortality, and serological data from Africa,<sup>70</sup> however, leaves substantial uncertainty in that region of the world. Because of the limited number of countries included in our overall and age-specific cumulative incidence estimates, we were unable to resolve differences between temperate and tropical countries. While data from Vietnam and Hong Kong were included and the incidence estimates – incidence in Vietnam was significantly lower possibly indicating differences in incidence in rural areas, and incidence from Hong Kong was consistent with incidence from the temperate countries included in our analysis – we are missing serologic data from many other low- and middle-income tropical and sub-tropical countries.

Our analysis demonstrates that approximately 24% of the populations of countries for which there are data were infected during the first wave of the pandemic, with incidence reaching 50% in school-age children. This meta-analysis offers a unique insight into the global impact of the 2009 influenza pandemic in its first year and highlights the need for seroepidemiological studies to be standardized and included in pre-pandemic preparedness plans. Together with estimates of global mortality,<sup>1,2</sup> our data have improved our understanding of the behavior and impact of the influenza pandemic of 2009.

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Conceived and designed the experiments: MDVK, AWM, SH. Analyzed the data: MDVK, AWM, SH, AK. ICMJE criteria for authorship read and met: MDVK, SH, AK, AWM, RA, DB, AB, AB, FB, MSC, MC, CYC, CMC, MC, NC, JC,

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Age-specific and overall cumulative incidence results including all available data (green) and excluding data from the US (Reed *et al.*, in press) and Norway (Waaen *et al.*, 2010).

**Table S1.** Pre-pandemic baseline H1N1pdm seroepidemiologic studies.

**Table S2.** H1N1pdm seroepidemiologic studies with pre- and post-pandemic sera used to calculate cumulative incidence.

**Table S3.** Post-pandemic single sera H1N1pdm seroepidemiologic studies.