Original Contribution

Age-Dependent Patterns of Infection and Severity Explaining the Low Impact of 2009 Influenza A (H1N1): Evidence From Serial Serologic Surveys in the Netherlands

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Despite considerable research efforts in specific subpopulations, reliable estimates of the infection attack rates and severity of 2009 influenza A (H1N1) in the general population remain scarce. Such estimates are essential to the tailoring of future control strategies. Therefore, 2 serial population-based serologic surveys were conducted, before and after the 2009 influenza A (H1N1) epidemic, in the Netherlands. Random age-stratified samples were obtained using a 2-stage cluster design. Participants donated blood and completed a questionnaire. Data on sentinel general practitioner-attended influenza-like illness and nationwide hospitalization and mortality were used to assess the severity of infection. The estimated infection attack rates were low in the general population (7.6%, 95% confidence interval: 3.6, 11) but high in children aged 5–19 years (35%, 95% confidence interval: 25, 45). The estimated hospitalization and mortality rates per infection increased significantly with age (5–19 years: 0.042% and 0.00094%, respectively; 20–39 years: 0.12% and 0.0025%; 40–59 years: 0.68% and 0.032%; 60–75 years: >0.81% and >0.068%). The high infection attack rate in children and the very low attack rate in older adults, together with the low severity of illness per infection in children but substantial severity in older adults, produced an epidemic with a low overall impact.

Abbreviations: CI, confidence interval; ICU, intensive care unit; ILI, influenza-like illness.

The 2009 influenza A (H1N1) pandemic illustrated that key aspects of influenza A virus epidemiology remain poorly understood. Early data on the pandemic suggested that the virus was highly transmissible (1). Consequently, much attention was focused on the possibility of an overwhelmed public health system, especially pediatric intensive care units (ICUs) (2). In hindsight, it is clear that these concerns did not materialize (3, 4).

A key ingredient to a better understanding of the epidemiology of influenza A epidemics is the infection attack rate, that is, the fraction of the population that has been infected during the epidemic. It is challenging to obtain reliable estimates of infection attack rates from surveillance of influenza-like illness (ILI) because of imperfect reporting and because many influenza A virus infections do not meet the definition of ILI or remain subclinical altogether. At present, serologic data collection is the best method of obtaining information on the true number of infections (2, 5–17; see reference 18 for a review). Although such serologic studies are an important advance over studies that focus on ILI only, relevant concerns remain. First, the pediatric and adolescent populations are underrepresented or absent in most studies, and samples have been taken from highly selected populations (hospitals, blood donors) (5, 7, 9, 11, 16). Second, most investigators have made use of convenience samples (6–10, 12, 19). Such study designs are opportune because of their timeliness and economic use of resources, but they also introduce bias (20). Moreover, serologic data cannot always

be linked to information from questionnaires (6–10, 12, 19), making it difficult to relate the findings to history of vaccination and ILI. Third, not all of the previous studies included a prepandemic control sample compiled from the same population as the pandemic sample (7–9, 13, 14, 16, 17, 19). This could hinder interpretation of the postpandemic data, because different populations may have varying degrees of preexisting immunity from earlier influenza A epidemics.

Here we present estimates of the age-specific infection attack rates for 2009 influenza A (H1N1) in the Netherlands, using population-based serial serologic surveys. Two age-stratified random samples were taken from the Dutch population, one before the epidemic occurred and the other afterward. We assessed the severity of 2009 influenza A (H1N1) infection by combining estimates of infection attack rates in unvaccinated participants with general practitioner consultations, laboratory-confirmed 2009 influenza A (H1N1) hospitalizations and ICU admissions, and laboratory-confirmed deaths.

MATERIALS AND METHODS

Study design and study population

Sustained transmission of 2009 influenza A (H1N1) in the Netherlands was documented from October 2009 (week 41) to December 2009 (week 51) (21). Vaccination started on November 9, 2009, and was recommended for children aged 6 months to 4 years, persons aged 60 years or older, and persons at elevated risk of developing severe disease. Two population-based surveys were conducted using 2-stage cluster sampling (Figure 1). Of 430 municipalities in the Netherlands, 38 were randomly selected, and an age-stratified random sample was drawn from the municipal population registers. For the first survey, conducted in September 2009, 2,970 persons were selected. For the second survey, conducted in March 2010–April 2010, 9,788 persons were selected.

For each selected person, information on age, sex, and municipality of residence was available from the population register. The selected individuals received an invitation to participate, an informed consent form, and a questionnaire. The questionnaire contained questions on demographic characteristics, living conditions, underlying illness, influenza vaccination history, history of ILI symptoms, and contact with persons with (potential) ILI. After returning the completed questionnaire, participants received a package with laboratory materials and were asked to donate blood at a local clinic. Participants received a gift voucher of €15 to cover their expenses.

The study was approved by the Medical Ethical Testing Committee of Utrecht University (Utrecht, the Netherlands), according to the Declaration of Helsinki.

Specimen collection and laboratory methods

Venous blood was collected in BD Vacutainer SST Advance Tubes (Becton, Dickinson & Company, Franklin Lakes, New Jersey). Depending on the degree of discomfort, finger-prick blood was obtained from young children, at a volume sufficient for hemagglutination inhibition testing. Blood was returned by regular overnight mail to the laboratory of the National Institute for Public Health and the Environment, and serum was stored at −20°C until analysis.

A hemagglutination inhibition assay was performed on the samples from both surveys using A/California/7/2009 (H1N1) (vaccine strain X-181) influenza virus as the hemagglutinating antigen. Turkey red blood cells were used as indicator cells. Serum samples were pretreated with cholera filtrate receptor-destroying enzyme to remove the autoagglutinating activity of the sera. After cholera filtrate treatment, 95 samples showed remaining autoagglutination. These samples were further pretreated by adsorption with packed turkey red blood cells. The pretreated samples were tested in duplicate at an initial (unstandardized) dilution of 1/20 in serial 2-fold dilutions. The international standard antiserum (pooled human serum against A/California/7/2009 (vaccine strain X-179A), obtained from the United Kingdom’s National Institute for Biological Standards and Control (catalog number 09/194) (22), was tested 12 times in duplicate and was used to convert titers to the international standard. Titers were expressed as the reciprocal of the highest dilution of serum which fully prevented hemagglutination. If the initial (standardized) dilution of 1/10.8 showed partial hemagglutination, the titer was set at


Figure 1. Geographic distribution of participants’ residences in 2 influenza A (H1N1) serologic surveys, the Netherlands, 2009–2010. The sizes of the circles reflect the number of serum samples collected per municipality (dark gray: prepandemic survey (September 2009); light gray: postpandemic survey (March/April 2010)).
1/5.4; when the 1/10.8 dilution did not show any inhibition, the serum was considered seronegative, and the standardized titer was set at 1/2.7. For each sample, the geometric mean value of the duplicate standardized titers was used.

**Data analyses and statistics**

Participants who did not return the questionnaire or failed to donate blood were excluded from further analysis. Non-responders were compared with responders on age using a Wilcoxon-Mann-Whitney test and on sex using a chi-square test.

To obtain estimates of the age-specific seroprevalence in unvaccinated persons, we determined the weighted proportion of persons with a standardized antibody titer greater than or equal to 1/40. Weights were calculated taking age and sex into account and were based on the Dutch population census of January 1, 2010. To address any uncertainty about the appropriate value for a cutoff defining seropositivity, we repeated the analysis using a mixture model (see Web Appendix (http://aje.oxfordjournals.org/)). This method does not specify a fixed cutoff but makes the less rigid assumption that an observed antibody titer belongs either to the seropositive distribution or to the seronegative distribution with a certain probability. The serologic infection attack rate was calculated as the difference in seroprevalence between the pre- and postpandemic surveys. The results presented below are based on the mixture analysis and are weighted on age. Full results, including estimates of the seroprevalence and infection attack rates obtained using the 1/40 cutoff, are presented in the Web Appendix.

The severity of infection in the Dutch population was defined as the age-specific probability per infection of consulting a general practitioner with ILI, hospitalization with laboratory-confirmed 2009 influenza A (H1N1), admission to an ICU, or death. The age-specific numbers of general practitioner consultations related to ILI between October 2009 (week 40) and April 2010 (week 17) were obtained from sentinel general practitioners (21). Because hospitalization or death involving laboratory-confirmed 2009 influenza A (H1N1) required notification in the Netherlands, the numbers of hospitalizations, ICU admissions, and deaths could be obtained from the national notification register (23).

Data analysis was performed using SAS 9.2 (SAS Institute Inc., Cary, North Carolina), R 2.11.1 (www.R-project.org; R Foundation for Statistical Computing, Vienna, Austria), and Mathematica 7.0 (Wolfram Research Inc., Champaign, Illinois).

**RESULTS**

**Study population**

The serologic samples provided adequate geographic coverage of the Netherlands (Figure 1). In the prepandemic survey, almost all serum samples had been drawn before the start of sustained community transmission (Figure 2). In this survey, serum samples from 367 persons were received, covering all age categories (Figure 3). The rate of response to the prepandemic survey was 12.4%. In the postpandemic sample, all sera were obtained after the epidemic had subsided. In this survey, 1,026 persons participated, yielding a response rate of 10.5%. In both surveys, responders were older than non-responders (median ages were 47 years and 37 years, respectively, in the prepandemic survey and 53 years and 46 years in the postpandemic survey; P < 0.001), and more women participated than men in both surveys (61% vs. 62%; P < 0.001).

**Sero prevalence and infection attack rates**

In both serologic surveys, the majority of sera from unvaccinated persons tested negative (81% and 70%), and there was substantial variation in hemagglutination inhibition titers in samples that tested positive (Figure 4). The distribution of titers showed a distinct bimodal pattern with clearly distinguishable seronegative and seropositive components (Figure 5). The titers from postpandemic survey participants tended to be higher than those from prepandemic survey participants (Figure 5), but this had a negligible effect on the classification of sera from the pre- and postpandemic surveys (Web Appendix).

Estimates of overall seroprevalence for the prepandemic survey were 19% (95% confidence interval (CI): 16, 22) using the mixture analysis and 7.0% (95% CI: 4.0, 10) using the cutoff of 1/40. Estimated seroprevalences did not differ between the sexes (Web Appendix) and appeared constant from age 20 years onwards (Figure 6A). In the prepandemic survey, no positive titers were observed in sera collected from children under age 10 years (Figure 6A).

Postpandemic survey estimates of overall seroprevalence in unvaccinated persons were 27% (95% CI: 24, 29) for the mixture analysis and 14% (95% CI: 10, 18) using the cutoff of 1/40. In the postpandemic survey, seroprevalence was high in the under-20 age groups (53%, 95% CI: 46, 61), lower in adults aged 20–39 years (28%, 95% CI: 21, 34), and still lower in those aged 40 years or older (16%, 95% CI: 14, 19) (Figure 6B).
Estimates of the overall infection attack rate were 7.6% (95% CI: 3.6, 11) using the mixture analysis and 7.3% (95% CI: 3.8, 11) using the cutoff of 1/40. The infection attack rate was highest in the age group 5–19 years, both in the mixture analysis (35%, 95% CI: 25, 45; Figure 6C) and when using the fixed cutoff (22%, 95% CI: 10, 33; Web Appendix). In adults aged 20–39 years, the attack rates were substantially lower (mixture analysis: 6.6% (<18 with 95% confidence); 1/40 cutoff: 7.3%, 95% CI: 3.0, 16), while the estimated infection attack rates were very low in the age categories of 40 years or older (mixture analysis: <2.8% with 95% confidence; 1/40 cutoff: 0.76% <5.0 with 95% confidence).

Severity of infection

Estimates of the severity of infection were obtained by dividing the age-specific incidence of general practitioner-attended ILI in the country and nationwide numbers of 2009 influenza A (H1N1)-related hospitalizations, ICU admissions, and deaths by the age-specific estimates of the incidence of infection (Table 1). Overall, this procedure yielded estimates of the percentages of 2009 influenza A (H1N1) infections resulting in a general practitioner consultation (21%, 95% CI: 14, 40), hospital admission (0.14%, 95% CI: 0.095, 0.27), ICU admission (0.017%, 95% CI: 0.015, 0.033), and death (0.0047%, 95% CI: 0.0032, 0.0092). There were significant differences in the severity of infection in different age groups, with substantially higher severity in persons over 40 years of age (Table 1). For instance, while the probabilities of hospitalization, ICU admission, and death were low for persons under 20 years of age, these increased more than 10-fold in persons aged 40 years or older.

DISCUSSION

Our data and analyses indicated that the infection attack rates of 2009 influenza A (H1N1) were much higher in children and adolescents than in adults, and that the estimated severity of infection was very low in children and moderate in older adults. Comparing our results with those of previous
studies shows that the estimated infection attack rates were similar to (6, 8, 15, 17, 24) or higher than (12, 13, 25) those previously reported in children (ages 5–19 years) and lower than those previously reported in older adults (ages ≥40 years) (5, 7, 8, 12, 15–17, 24). The combination of high incidence with low severity in children and very low incidence with moderate severity in older persons yielded a pandemic with a low overall impact. Therefore, the observed sharp contrasts between the age distributions for infection attack rate and severity solve an apparent paradox: The 2009 influenza A (H1N1) epidemic had a modest impact in terms of use of hospital and ICU capacity, despite the fact that attack rates were higher than those for seasonal influenza, while the virus was not unusually avirulent (3, 4, 26, 27).

The observations for the 2009 influenza A (H1N1) pandemic differ from those of previous pandemics (1918 Spanish flu, 1957 Asian flu, 1968 Hong Kong flu) in which high clinical attack rates and mortality were observed in the younger population (28, 29). Quantitative comparison of the estimates for severity of infection with results from other studies on 2009 influenza A (H1N1) are problematic, because different studies have used different denominators for calculating the risks of hospital admission and death. Earlier estimates of the probability of hospitalization due to 2009 influenza A (H1N1) infection ranged from 0.1% (1, 4, 16, 17, 30) to 0.9% (15, 31). The estimated probability of death upon infection in the 2009 pandemic ranged from 0.004% to 0.4% (1, 4, 15–17, 30, 31). The qualitative age pattern for severity, with a low risk of dying for young persons and a moderate risk for older persons, is consistent with previous reports (4, 15, 30). The apparent age-specific pattern of hospitalization that we observed was less clear in earlier reports.

**Figure 5.** Relative frequency distributions of influenza A (H1N1) hemagglutination inhibition titers before and after the 2009 epidemic, the Netherlands, 2009–2010. Dark gray bars, participants in the pre-pandemic survey; light gray bars, unvaccinated participants in the postpandemic survey.

**Figure 6.** Influenza A (H1N1) seroprevalence and infection attack rates in the Netherlands, by age group, 2009–2010. A) Estimated age-specific seroprevalence in the prepandemic survey. Dark gray areas, 95% confidence interval. B) Estimated age-specific seroprevalence in unvaccinated participants in the postpandemic survey. Medium gray areas, 95% confidence interval. C) Estimated age-specific serologic infection attack rates. Infection attack rates were calculated as the difference in seroprevalence between the post- and prepandemic surveys. Light gray areas, 95% confidence interval.
Table 1. Severity of Infection With 2009 Influenza A (H1N1), the Netherlands, 2009–2010

<table>
<thead>
<tr>
<th>Age Group, years</th>
<th>1–4</th>
<th>5–19</th>
<th>20–39</th>
<th>40–59</th>
<th>60–75</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of population</td>
<td>4.5</td>
<td>25</td>
<td>&lt;18</td>
<td>&lt;6.9</td>
<td>&lt;0.8</td>
<td>7.6</td>
</tr>
<tr>
<td>Infection attack rate</td>
<td>NA*</td>
<td>35</td>
<td>6.6</td>
<td>1.3</td>
<td>NA</td>
<td>7.6</td>
</tr>
<tr>
<td>ILI-related GP consultations</td>
<td>5.3</td>
<td>2.1</td>
<td>1.3</td>
<td>1.2</td>
<td>1.0</td>
<td>1.6</td>
</tr>
<tr>
<td>No. of hospitalizations</td>
<td>269</td>
<td>442</td>
<td>332</td>
<td>428</td>
<td>167</td>
<td>1,638</td>
</tr>
<tr>
<td>No. of ICU admissions</td>
<td>7</td>
<td>38</td>
<td>37</td>
<td>78</td>
<td>37</td>
<td>197</td>
</tr>
<tr>
<td>Severity of infection, % per infection</td>
<td>5.8</td>
<td>4.6, 8.2</td>
<td>&gt;7.3</td>
<td>93</td>
<td>&gt;18</td>
<td>NA</td>
</tr>
<tr>
<td>ILI-related GP consultations</td>
<td>NA</td>
<td>0.042</td>
<td>0.033, 0.059</td>
<td>&gt;0.044</td>
<td>0.68</td>
<td>&gt;0.13</td>
</tr>
<tr>
<td>Hospitalizations</td>
<td>NA</td>
<td>0.0036, 0.0051</td>
<td>0.013</td>
<td>&gt;0.0047, 0.012</td>
<td>&gt;0.0023</td>
<td>NA</td>
</tr>
<tr>
<td>ICU admissions</td>
<td>NA</td>
<td>0.00094, 0.0013</td>
<td>0.0025</td>
<td>&gt;0.00093, 0.0032</td>
<td>&gt;0.00060</td>
<td>NA</td>
</tr>
<tr>
<td>Deaths</td>
<td>NA</td>
<td>0.00004, 0.00027</td>
<td>0.0003</td>
<td>&gt;0.00024, 0.00047</td>
<td>&gt;0.00018, 0.00047</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; GP, general practitioner; ICU, intensive care unit; ILI, influenza-like illness; NA, not available.

a All participating children under 5 years of age were vaccinated; therefore, the infection attack rate could not be estimated.

b No point estimate was available because the estimated postpandemic prevalence was lower than the estimated prepandemic prevalence.

c For 47 hospital admissions, it was unknown whether ICU admission was required.
Although our conclusions are in broad agreement with previous studies, several factors may have affected our estimates. First, estimates of infection attack rates and severity might have been affected by selective response. However, because data on the ages and sexes of nonresponders were available, it was possible to detect selective nonresponse and correct for it. Detection of selective response with respect to underlying risk factors proved more challenging. It is recommended that persons with underlying risk factors be vaccinated against seasonal influenza; thus, one would expect that selective response manifests itself as a difference between the seasonal influenza vaccination coverage among survey participants and that in the general Dutch population (32). In an additional analysis, however, no such difference was observed. It is therefore unlikely that selective response affected our estimates of 2009 influenza A (H1N1) infection attack rates and severity.

Second, imperfect reporting and differential (age-specific) health-care-seeking behavior could have affected our estimates of the severity of infection. Additionally, the moderate correlation between ILI and influenza infection could have led to overestimation of the probability of general practitioner-attended ILI upon infection, since there is no guarantee that ILI is caused by influenza A (H1N1) infection. This is arguably less of a problem for influenza-related hospitalizations, ICU admissions, and mortality, because 2009 influenza A (H1N1) hospitalizations and deaths remained notified throughout the epidemic, and all hospitalizations were laboratory-confirmed. However, age-dependent referral patterns could have influenced hospitalization and ICU admission rates. In all, we believe that severity estimates based on ILI may be biased, that severity estimates based on fatalities may be imprecise because of small numbers, and that comparisons of severity of infection between different age groups are best based on hospitalization or ICU data.

Third, at the time of the prepandemic survey, the 2009 influenza A (H1N1) virus had already begun to circulate endemically in the Netherlands (Figure 1). This indicates that a number of early 2009 influenza A (H1N1) infections may have been included in the prepandemic survey, thereby inflating the prepandemic prevalence and suppressing estimated infection attack rates. In our prepandemic survey, 90% of the serum samples were collected at a time when only 10% of all hospitalizations had occurred. Assuming that hospitalizations reflect the incidence of infection in the population, this suggests that the probability of including infections in the prepandemic survey was very small. Moreover, we found no trend of increasing positive titers over the course of the prepandemic survey, which again suggests that even if some persons who were seropositive for 2009 influenza A (H1N1) early in the outbreak were included in the prepandemic survey, the impact would have been small.

One could ask why the pandemic was so strongly restricted to the younger age groups. One possible answer is that differences in contact patterns between age groups were responsible for differences in infection attack rates between age groups (33, 34). Model predictions based on observed age-specific contact patterns suggest that part of the observed differences in attack rates between age groups can be accounted for (data not shown). However, these predictions also indicate that the extreme differences in attack rates between younger and older persons (Figure 6C) cannot be explained solely by different contact patterns, unless a considerable fraction of older persons had protective immunity. This explanation has received some support in recent molecular and epidemiologic studies (12, 35–37).

One would expect future epidemics of 2009 influenza A (H1N1) to have a flatter age profile of infection incidence than was observed in 2009, with younger age strata being less dominant and lower overall infection attack rates. This has been observed in previous pandemics (28, 38). Our findings further suggest that the overall severity of a pandemic is hard to predict, as even a small increase in the infection attack rate in older persons could drastically increase the numbers of persons needing hospitalization and ICU treatment. We conclude that monitoring of the potential of the influenza A (H1N1) virus to be transmitted in groups with a high risk of a severe outcome is essential in pandemic and postpandemic planning.

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