The Study

A total of 342 healthy adult staff and students of Imperial College London (London, UK) were recruited during September–November 2009 and followed for 2 consecutive influenza seasons: 2009–10 and 2010–11 (Figure 1). Participants’ median age was 28 years (interquartile range 20–36 years); 83% were <40 years of age. At each time point, collected serum samples were tested for antibodies to A(H1N1)pdm09 virus (A/England/195/09 strain) by the hemagglutination-inhibition (HI) assay (4). Participants were asked to record temperature, self-sample, and return nasal swabs when experiencing influenza-like symptoms. Swabs were tested for respiratory viruses with standardized real-time reverse transcription PCR. Influenza seroprevalence rates were defined as the proportion of persons with HI titers $\geq 32$ (4).

Because our study began at the end of the first pandemic wave, cumulative incidence of A(H1N1)pdm09 infection over the first wave was estimated as the difference between age-specific seroprevalence rates at recruitment ($T_0$ in Figure 1) and published prepandemic (2008) seroprevalence rates for England (4). Incident infection was defined as antibody seroconversion (4-fold rise in HI titer) in paired serum samples collected at the start and end of a wave among unvaccinated persons (because HI assay cannot differentiate infection from vaccination) or detection of A(H1N1)pdm09 virus in nasal swabs. The incidence of infection was estimated for the second and third waves as the proportion of incident infections among unvaccinated participants.

Development of any symptoms was recorded on a Web-based questionnaire emailed to participants every 3 weeks. The average response rate was 75%. Illness episodes were categorized as acute respiratory infection (episode with any symptoms), influenza-like illness (ILI episode with fever plus cough or sore throat), and fever (recorded temperature $\geq 38^\circ$C) alone. Visits to primary care or hospital during illness were also recorded. Data were analyzed using Stata version 9.0 (StataCorp, College Station, TX, USA) with the $\chi^2$ test to compare proportions and $t$ test to compare means after checking for normal distribution by assessing for kurtosis, skewness, and the Shapiro-Wilk test. Hosmer-Lemeshow test was used to estimate goodness-of-fit for each logistic regression.

At recruitment, after the first pandemic wave, A(H1N1)pdm09 seroprevalence was 26% (95% CI 21.4–31.2), with seroprevalence significantly higher in participants 18–25 years of age than in older age groups (Table 1). Participants with ILI in the preceding 3 months corresponding to the first wave had significantly higher ($p<0.001$) mean A(H1N1)pdm09 virus HI titers, which in conjunction with the age distribution, suggests first-wave infection rather than cross-reactive antibodies (5). Overall cumulative incidence
Incidence of Influenza A(H1N1)pdm09 Infection

during the first wave was 12.7% (95% CI 7.1%–18.4%) and 26.6% (95% CI 15.3%–37.8%) among participants 18–25 years of age with no increase in older age groups (online Technical Appendix Table 1, wwwnc.cdc.gov/EID/ articlepdfs/19/11/13-0295-Techapp1.pdf).

The incidence of infection over the third pandemic wave was significantly higher (p = 0.02) than over the second wave (Figure 1). Among participants with prewave titers <8, the incidence of infection was significantly higher over the third wave than over the second wave (p<0.001); incidence did not differ for participants with prewave titers >8 (Table 2, Appendix, wwwnc.cdc.gov/EID/article/19/11/13-0293-T2.htm). Age-specific incidence was significantly higher (p = 0.01) over the third wave than the second wave among participants 26–40 years of age (third wave: 25.4% [95% CI 15.2–35.5]; second wave: 10.9% [95% CI 5.1–16.7]) but not the other age groups (Table 2, Appendix). For 11 infected participants with paired serum samples and virus detected in nasal swabs, 2 (18%) did not show antibody seroconversion (online Technical Appendix Table 2).

During an illness episode, 20% of infected participants reported fever or ILI, 17% visited their general practitioner, and none visited a hospital (Figure 2). Because predictions

![Graph showing incidence and visits over waves](image-url)

Figure 1. Incidence of natural influenza A(H1N1)pdm09 infection in the study cohort during the 3 pandemic waves in context of the evolving pandemic, United Kingdom. Study outline is depicted in the upper panel in temporal context of the pandemic during the 2009–2011 influenza seasons. The bar chart shows UK influenza virologic surveillance data from WHO Flunet (www.who.int/influenza/gisrs_laboratory/flunet/en/) highlighting the periods of study recruitment and follow-up in relation to influenza A activity in the United Kingdom during 2009–2011. Light gray bars indicate influenza A of all subtypes; dark gray bars indicate the number of A(H1N1)pdm09 cases detected by virologic national surveillance. Healthy adults were recruited after the first pandemic wave (April–August 2009) had ended in the United Kingdom and were followed over 2 influenza seasons, with serum samples collected before and at the end of each influenza season. The median time between visits is shown. The second wave was defined as baseline (September–November 2009) to first follow-up (February–April 2010) and the third wave as the time between the second follow-up (August–November 2010) and the third follow-up (February–April 2011). The light gray bracket and numerals represent the estimated cumulative incidence of infection over the first pandemic wave by calculating the difference between and seroprevalence rates at baseline in the cohort and prepandemic (2008) published seroprevalence rates. Infection was defined as detection of A(H1N1)pdm09 virus in nasal swabs returned during the second or third wave or a 4-fold rise in A(H1N1)pdm09 virus HI titer in paired serum samples collected at the start and end of each wave. The number of infected persons with total persons at risk during each of the second and third waves with calculated incidence rate and 95% CIs are shown. WHO, World Health Organization; IQR, interquartile range; HI, hemagglutination-inhibition. *Infection rates in the first wave reflect cumulative incidence of infection, estimated by calculating the difference in proportion of persons with HI titer >32 between baseline (T₀) and published Health Protection Agency data before the pandemic in 2008.
of a small third pandemic wave were disproved (4), the reasons for this large wave remained unclear. Multivariate logistic regression was undertaken with infection as the dependent variable and age, sex, and prevaccine titers as independent variables. Each doubling increase in prevaccine HI titers, after adjustment for age and sex, was associated with increased risk for A(H1N1)pdm09 infection associated with protection against symptomatic illness (14). Incidence in our cohort was lower than that estimated for England by cross-sectional sera surveys (7,11). This finding may reflect our accounting for individual-level vaccination status and baseline antibody titers; data usually unobtainable with cross-sectional population-sample sera surveys. However, our study did not include children or elderly persons, which limits the generalizability of our findings. A major advantage of longitudinal cohort studies recording clinical data is identification of subclinical and asymptomatic infections. More than 80% of participants did not seek primary care or have surveillance-defined ILI as symptomatic. More than 80% of participants with influenza reported no symptoms. Cross-reactive cellular immune responses that are highly prevalent in the population (13) have recently been shown to be associated with protection against symptomatic illness (14). Our analysis of pandemic influenza in a community cohort over successive seasons offers insight into of a small third pandemic wave were disproved (4), the reasons for this large wave remained unclear. Multivariate logistic regression was undertaken with infection as the dependent variable and age, sex, and prevaccine titers as independent variables. Each doubling increase in prevaccine HI titers, after adjustment for age and sex, was associated with increased risk for A(H1N1)pdm09 infection associated with protection against symptomatic illness (14). Incidence in our cohort was lower than that estimated for England by cross-sectional sera surveys (7,11). This finding may reflect our accounting for individual-level vaccination status and baseline antibody titers; data usually unobtainable with cross-sectional population-sample sera surveys. However, our study did not include children or elderly persons, which limits the generalizability of our findings. A major advantage of longitudinal cohort studies recording clinical data is identification of subclinical and asymptomatic infections. More than 80% of participants did not seek primary care or have surveillance-defined ILI as symptomatic. More than 80% of participants with influenza reported no symptoms. Cross-reactive cellular immune responses that are highly prevalent in the population (13) have recently been shown to be associated with protection against symptomatic illness (14). Our analysis of pandemic influenza in a community cohort over successive seasons offers insight into
contributers of the unexpectedly larger third pandemic wave. Our analysis also highlights the necessity of using cohorts to complement routine case-based surveillance to estimate influenza burden.

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References

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Figure 2. Proportion of influenza A(H1N1)pdm09–infected persons who had symptoms during their illness episode during the second wave (September 2009–April 2010), third wave (August 2010–April 2011), and entire study period, United Kingdom. Proportion of persons with reported symptoms over the study period is combined from the second and third waves. Symptoms were recorded by a Web-based symptom questionnaire emailed to participants every 3 weeks. Symptoms associated with illness episode were acute respiratory infection (ARI; illness episode with any symptoms), influenza-like illness (ILI; episode with fever plus cough or sore throat), fever (recorded temperature ≥38°C) alone, or visit to a general practitioner (GP). The graph depicts the average with 95% CIs calculated by using binomial distribution.