Published in final edited form as:
Int J Infect Dis. 2011 November ; 15(11): e781–e786. doi:10.1016/j.ijid.2011.07.002.

Seroprevalence of antibodies to influenza A/H1N1/2009 among transmission risk groups after the second wave in Mexico, by a virus-free ELISA method

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Summary

Objective—No serological studies have been performed in Mexico to assess the seroprevalence of influenza A/H1N1/2009 in groups of people according to the potential risk of transmission. The aim of this study was to determine the seroprevalence of antibodies against influenza A/H1N1/2009 in subjects in Mexico grouped by risk of transmission.

Methods—Two thousand two hundred and twenty-two subjects were categorized into one of five occupation groups according to the potential risk of transmission: (1) students, (2) teachers, (3) healthcare workers, (4) institutional home residents aged >60 years, and (5) general population. Seroprevalence by potential transmission group and by age grouped into decades was determined by a virus-free ELISA method based on the recombinant receptor-binding domain of the hemagglutinin of influenza A/H1N1/2009 virus as antigen (85% sensitivity; 95% specificity). The Wilson score, Chi-square test, and logistic regression models were used for the statistical analyses.

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Ethical approval: Approval was obtained from the ethics and research committees of the School of Medicine TEC de Monterrey and the Education and Health secretariats. Written informed consent was obtained from all subjects and from parents of those aged younger than 18 years.

Conflict of interest: The authors have nothing to disclose; no financial or other conflicts of interest.

Results—Seroprevalence for students was 47.3%, for teachers was 33.9%, for older adults was 36.5%, and for the general population was 33.0%, however it was only 24.6% for healthcare workers ($p = 0.011$). Of the students, 56.6% of those at middle school, 56.4% of those at high school, 52.7% of those at elementary school, and 31.1% of college students showed positive antibodies ($p < 0.001$). Seroprevalence was 44.6% for college teachers, 31.6% for middle school teachers, and 29.8% for elementary school teachers, but was only 20.3% for high school teachers ($p = 0.002$).

Conclusions—The student group was the group most affected by influenza A/H1N1/2009, while the healthcare worker group showed the lowest prevalence. Students represent a key target for preventive measures.

Keywords

Seroprevalence; Antibodies; A/H1N1/2009; Pandemic influenza; Risk group

Introduction

In April 2009, a new pandemic strain of influenza infected thousands of persons in Mexico and the USA, and spread rapidly throughout the world.[1,2] A second wave swept through Mexico from October to December 2009. As of July 2010, more than 214 countries worldwide had reported more than 1 million laboratory-confirmed cases of pandemic influenza A/H1N1/2009,[3] while Mexico had reported 72 548.[4] However, the true number in Mexico has not yet been determined due to a lack of serological evidence, which might have resulted in an underestimation of the true infection rates in the population. Reasons for under-ascertainment include: asymptomatic cases, not all ill persons seek medical care and have a specimen collected, not all specimens are sent for confirmation with reverse transcription PCR (RT-PCR), and cases of negative results because of the timing of collection or the quality of the specimen.[5,6]

Most of the estimations of the prevalence of the influenza A/H1N1/2009 pandemic have been performed using indirect measures and predictions, such as multiplier probabilistic models based on laboratory-confirmed cases,[7] serum cross-reactive antibody responses to infection[8] or after vaccination with seasonal influenza vaccine,[9,10] computational approaches,[11] or estimations derived in other countries from the number of cases imported from Mexico.[12,13]

Some have identified seroprevalence against pandemic H1N1 in different countries: 32% for children younger than 15 years of age and 20% for those aged 15–24 years in England,[6] 5.6% in Guangxi Province, China, [9] 21% in Pittsburg, PA, USA,[14] from 27.7% to 42.8% in Scotland,[15] and 13% in Singapore.[16] Other authors have studied particular groups and have found a seroprevalence of 20% in hospital staff in Singapore,[17] or have measured the antibody response to the pandemic virus resulting from previous influenza infection or vaccination.[14,18]

Without a direct serological measure, predictions are subject to substantial uncertainty. Direct measurement of the seroprevalence provides valuable information and reliable figures

about the epidemiology of the infection, and may be useful in decision-making about transmission models, immunization strategies, and policy-making processes.[5,11]

No serological studies have been performed in Mexico to assess the seroprevalence of influenza A/H1N1/2009 in groups of people according to the potential risk of transmission. Therefore, this cross-sectional study aimed to identify seroprevalence of antibodies to pandemic influenza A/H1N1/2009 in a large population, separated into groups by potential risk of transmission according to occupation, at the end of the second wave of the pandemic in Mexico. The goal was to provide a direct measure of the incidence of infection in these groups, assessed indirectly by the seroprevalence.

Materials and methods

Subjects

This was a cross-sectional study of 2222 subjects whose serum samples were collected between November 9 and December 17, 2009 in the metropolitan area of Monterrey, in northeastern Mexico. The region has a population of 2 708 529, including 717 155 students,[19] 40 823 teachers,[20] and 234 213 adults over 60 years of age.[21] Subjects were categorized into one of five groups and subgroups, according to the potential risk of transmission of the influenza A/H1N1/2009 virus, depending on occupation. Table 1 shows the number, proportion, and age of the subjects in each group. An open invitation to participate voluntarily in the study was made to the community. Blood samples were drawn on site for the different groups: six elementary and middle schools, four high schools, one university, four institutional homes for older adults, and three hospitals; however samples from the general population group came from all over the metropolitan area and were collected at a single site. Inclusion criteria were voluntary participation and overnight fasting. Through face-to-face interviews, subjects were asked if they had received seasonal influenza A 2008, 2009, or pandemic influenza A/H1N1/2009 vaccinations.

Measurement of antibodies to influenza A/H1N1/2009

Overnight fasting blood samples were drawn from subjects, centrifuged within 3 h, and frozen at −80 °C.

A virus-free ELISA method,[22] based on the recombinant receptor-binding domain of the hemagglutinin of influenza A/H1N1/2009 virus as antigen, was employed to determine specific antibody titers against pandemic influenza virus in serum samples. A solution of mouse anti-histidine tag antibodies (AbD Serotec, UK) in phosphate-buffered saline (PBS) was dispensed into microassay plate wells (Maxisorp, Corning Inc., USA), incubated, and then repeatedly washed. A blocking buffer (SuperBlock T20 PBS; Cat. No. 37516, Pierce Biotechnology, USA) was added to block the surface not covered with antibodies, and the wells were then washed again. A non-glycosylated, histidine-tagged recombinant protein fragment of the hemagglutinin of influenza A/H1N1/2009 virus, expressed in *Escherichia coli*,[23] was then added. The proper folding of this protein was demonstrated by X-ray crystallography according to DuBois et al.[24] The solution was incubated and then washed. To test for specific bio-recognition, 100 μl of the serum sample to be assayed (1:50 in PBS)

was added to each well, incubated, and repeatedly washed. To reveal the amount of antibody specifically bound, 100 μl/well of an anti-human IgG antibody solution (1:30 000 dilution in PBS–Tween 0.05%) marked with horseradish peroxidase (Pierce Biotechnology) was used. After incubating and washing, 100 μl/well of substrate solution (1 Step Ultra TMB-ELISA; Lot. 34028, Pierce Biotechnology) was added. After incubation the enzymatic reaction was stopped by adding 50 μl/well of 1 M H_2SO_4. The color produced by the enzymatic reaction was evaluated by absorbance at 450 nm with a Biotek microplate reader (Biotek, USA). Absorbance values were normalized for each plate based on the signal of serum from one or several subjects not exposed to influenza A/H1N1/2009. For this study, serum samples with normalized absorbance values above 2.0 were considered seropositive for influenza A/H1N1/2009 virus. This threshold value is considered conservative and minimizes the possibility of false-positive samples, since typical normalized absorbance values from non-exposed individuals ranged between 1.0 ± 0.25 (mean ± 1 standard deviation).[22]

From the entire population studied, 950 subjects (mean age 40.8 years) claimed that they had been vaccinated against seasonal influenza 2008 and/or 2009, and were tested for cross-reactivity with the recombinant protein used as antigen in the ELISA assay.

In order to compare the diagnostic performance of the ELISA method used here against standard methodologies, particularly hemagglutination inhibition (HI) assays, an additional set of 20 serum samples from PCR-positive convalescent influenza A/H1N1/2009 patients and 20 non-exposed subjects (samples collected during the year 2008, before the influenza A/H1N1/2009 pandemic onset) were analyzed both by ELISA (samples diluted 1:50 in PBS) and HI assays. Positive volunteers were recruited from regular patients at Hospital San José Tecnológico de Monterrey and Clínica Nova during October and November 2009. Samples were taken between 2 and 24 weeks after infection. HI assays were conducted at the Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, TN, USA, according to standard methodologies.[22]

Statistical analysis

Sample size was calculated using a 95% confidence level and a desired confidence interval of 3%. The estimated study proportion was 30%, according to the seroprevalence in probable cases of influenza A/H1N1/2009 reported in Mexico by mid 2009.[25]

The prevalence of influenza A/H1N1/2009 seropositivity is presented as the percentage of individuals with positive antibodies tested in each group with its 95% confidence interval in parenthesis. We used the Wilson score method to obtain the confidence intervals. Differences in proportions were evaluated by Chi-square tests. Multivariate analysis was performed by logistic regression models (backward, stepwise) with the presence or absence of antibodies as a dependent dichotomous variable and adjusting for risk of transmission groups, age groups (age grouped into decades), and gender where appropriate. The resulting models were found adequate by the Hosmer–Lemeshow goodness of fit test. Multicollinearity between occupation risk and age was evaluated through calculation of the corresponding variance inflation factor (VIF) value. The gender variable was found not significant in all models. A two-tailed *p*-value of <0.05 was considered statistically

significant. All analyses were performed with SPSS Statistics 17.0 software (IBM-SPSS, USA).

Results

There was a significant difference in the percentage of serum samples that tested positive for antibodies to pandemic influenza A/H1N1/2009 virus among the diverse risk groups. Students had the highest seroprevalence (47.3%), followed by older adults (36.5%), while healthcare workers had the lowest (24.6%). The result for the VIF for multicollinearity between occupation risk and age was 2.4, which represents a low multicollinearity (Table 1). The percentage of persons who tested positive for influenza A/H1N1/2009 was significantly higher for the middle and high school student groups (56.6% and 56.4%, respectively), followed closely by the elementary school children group. Seroprevalence for the elementary and middle school teacher groups (29.8% and 31.6%, respectively) was significantly lower than that of their corresponding student groups (Chi-square p-value < 0.001). The lowest percentage was observed for high school teachers (20.3%), while a striking increase in seroprevalence was noted for college teachers (44.6%), which was even higher than for college students (31.1%) (Table 2).

None of the participants had been vaccinated against influenza A/H1N1/2009, while 28.0% had received seasonal vaccination for influenza A 2008 and 33.5% for seasonal influenza A 2009.

Regarding age cohorts, grouped in decades, the percentage of persons who tested positive for antibodies against influenza A/H1N1/2009 was highest in the 6–10 years group (51.1%), followed closely by the 11–20 years group (49.0%). A decreasing seropositive trend was seen as age increased up to those aged 60 years (25.2%), but then an increase in seroprevalence was seen for those >60 years of age, reaching 41.0% in those aged 71–80 years (Table 3). The Chi-square test showed there was a significant difference in the proportions of seroprevalence for the different age cohorts ($p < 0.000$).

The percentage of positive samples from the 950 subjects vaccinated against seasonal influenza 2008 and/or 2009, according to ELISA, was 35.1%, which was not significantly different to the percentage of the non-vaccinated subset, at 41.4% ($p = 0.139$, adjusted for sex and age). None of the 950 subjects tested showed cross-reactivity with the recombinant protein used as antigen in the ELISA assay.

The sensitivity and specificity of the ELISA method, considering a threshold value of normalized absorbance of 2.0, were 85% and 95%, respectively. In the comparative analysis of the ELISA method and HI assays, the ELISA method determined 85% of the positive cases as such, while only 50% of the positive cases were precisely diagnosed by HI (when the conventional threshold of 1:40 dilution for agglutination inhibition was considered an indicator of seropositivity). More details on the comparative performance of the ELISA method used here and the conventional HI assay is presented elsewhere.[22,26]

The ELISA method used here yields adequate reproducibility and a high signal/noise ratio within determinations in the same microplate and among different microplates.[27] Using a

normalized absorbance value of 2.0, the method was able to discriminate samples from convalescent patients, preferably after the third week of infection, and at least up to the 24th week of exposure. Assay sensibility was further validated against results from HI assays. A previous report showed that all members in a pool of 14 samples diagnosed as positive by HI exhibited normalized absorbance values higher than 1.5, and 85% of them exhibited normalized absorbance values higher than 2.0.[22] In general, high HI titers (>1:320) were correlated with normalized absorbance values higher than 4.0. In addition, the ELISA method and the HI assay were used to diagnose a pool of 17 serum samples corresponding to convalescent H1N1/2009 patients diagnosed by RT-PCR. All samples determined as positive by HI (10 samples) were also positive by ELISA. While sensitivity of the HI assay was 10/17 = 58.88% (using a positivity criterion of inhibition at dilutions higher or equal to 1:40), the ELISA method recognized 100% of samples as positive when a threshold of 1.5 was used, and 85% of samples as positive when a threshold of 2.0 was used.[27] With this very same threshold, 3.88% of false-positives were observed when 100 serum samples from non-exposed individuals (samples collected in 2008, before the onset of the pandemic) were used.

Discussion

The influenza A/H1N1/2009 virus has resulted in the first influenza pandemic in more than four decades.[28] A need for more comprehensive serosurveys to understand infection rates and population immunity has emerged, since relying on laboratory-confirmed cases limits the ability to understand the full impact and severity of the epidemic.[29] This study, which examined real-time seroprevalence at the end of the fall wave in Mexico,[30,31] contributes to our understanding of the spread of the pandemic throughout the population. It may also explain some of the differential distributions not only of affected age groups, but particularly of certain risk groups, according to potential risk of infection with the virus. To our knowledge, this is the first study of this type.

The results of this study of 2222 people indicate an indirect sign of infection of specific risk groups according to the seroprevalence found. We found no difference in the seroprevalence between genders. The proportion of people with positive antibodies to influenza A/H1N1/2009 virus was significantly higher for students as a group (47.3%), followed by teachers (33.9%), and closely by the general population (33.0%), while that of healthcare workers was the lowest (24.6%). Interestingly, teaching students (high school) with a high seroprevalence (up to 57%) seems to be associated with a low seroprevalence (down to 30%). Even though their respective mean ages were similar, 42.0 ± 10.3 years for teachers and 40.6 ± 11.9 years for the general population, college teachers showed a significantly higher seroprevalence (44.6%) compared to the general population (33.0%) (Chi-square p = 0.013), which might be due to their close exposure to students (Table 1). We also have to consider that the different prevalence rates in the teachers in contact with diverse student groups may reflect baseline differences in the prevalence of cross-reactive antibodies. Intense preventive measures and increased awareness might account for the lowest prevalence in the healthcare workers group. Older adults living in institutional homes showed a prevalence of 36.5%, which might be explained by previous exposure to a 1918-like H1N1 virus, as has been documented.[5,6,18,28,32] The general population group included

people from diverse occupations with varied contact and socialization patterns, which might have placed them at lower risk. However, we have to consider that data from such a heterogeneous group are difficult to interpret.

Students are more predisposed to transmission and spread of the virus because of their greater close contact within limited classroom spaces for 6–8 h/day. This is particularly the case for elementary and middle school students, which might explain their strikingly higher seroprevalences (52.7% and 56.6%, respectively). High school students showed a higher prevalence (56.4%) compared to college students (31.1%). This might be explained by the nature of socialization outside of school and by greater contact during sports and cultural activities within the school. These findings are consistent with the high level of susceptibility in children and adolescents, and the increased potential for acquisition and subsequent transmission of influenza that occurs within schools.[33] The milder disease seen with this pandemic may also have contributed to its increased spread.[5,18]

Concerning seroprevalence according to age grouped into decades (Table 3), there was a significantly higher prevalence of antibodies to influenza A/H1N1/2009 in those aged 6–10 years (51.1%) and 11–20 years (49%), with a decreasing tendency apparent as age advanced up to those aged 51–60 years (25.2%), but then rising again in those >60 years (35.6–41.0%). Half of those in the population aged 20 years or younger were seropositive and the proportion was almost double that of people aged 31–60 years. From April 2009 to December 2009, there were 67 982 confirmed cases/800 deaths in Mexico, distributed by age group as follows: 0–4 years: 7447/54; 5–9 years: 10 496/36; 11–20 years: 19 771/48; 21–30 years: 12 950/156; 31–40 years: 7261/182; 41–50 years: 5063/146; 51–60 years: 3082/135; 61–70 years: 1280/43; non-specified: 631/0. In our study participants, the prevalence was highest in those aged 5–9 years (51.1%), followed by the 10–19 years group (49.0%), then by the 20–29 years group (37.1%), declining thereafter. Approximately 70% of the deaths occurred in those aged 20–55 years. Compared to the incidence rates of confirmed cases that occurred among those aged 5–10, 11–20, and 21–30 years, our study participants aged 5–9 years had the highest seropositivity rate. This might indicate that this particular very young group suffered from asymptomatic transmission more than the other groups. Immunization strategies in this group of the young and susceptible should be reinforced to reduce transmission.

Our data showed a higher prevalence for all age and cohort groups than has been reported from other countries, such as the USA,[5,14] Singapore,[16] and England,[6] but were similar to findings from Scotland.[15] Similarly, prevalence in older adults has differed greatly.[9,18,32,34,35] This higher prevalence in Mexico might partly be explained by the timing of the epidemic. The first reported cases of confirmed influenza A/H1N1/2009 occurred in Mexico in April 2009. At the time, a lack of awareness might have resulted in infection of a greater proportion of the population during the first wave, since no preventive measures were applied until about 1 month later. In contrast, in other countries, preventive measures were applied prior to the onset of the epidemic. We also have to consider that studies from these countries used the conventional HI method for measuring strain-specific influenza antibodies, and our results derive from the ELISA-based method described.

To estimate the incidence of 2009 pandemic H1N1, several approaches have been used that combine data from established surveillance systems and from mathematical and computational models.[7,11,36–38] Estimates such as these probably underestimate the total number of people infected. One study determined the reference range for the number of cases in Mexico: 121 000 to 1 394 000.[11] Two other studies reported a ratio of infected cases in Mexico as low as 1 in 100, derived from infected travelers, which are orders of magnitude larger than those confirmed by the Mexican authorities.[12,13]

The region we assessed has a population of 2 708 529, including 717 155 students,[19] 40 823 teachers,[20] and 234 213 adults over 60 years.[21] There are no data in Mexico for the seroprevalence in the community after the first wave, nor are there data for the possible rate of waning of antibodies acquired at that time, in order to estimate if immunity gained in the first wave could have persisted at the time of the present study. A between-wave collection would have been desirable. Although we do not have information on baseline seropositivity to calculate the actual attack rate, our results might be an indication that the number of confirmed cases in Mexico could be a gross underestimation of the actual number of infections.[39] Other countries have demonstrated an underestimation of cases as well.[12,13,40] This highlights the usefulness of serosurveys for a more complete understanding of the extent of the infection with the pandemic virus,[41] and for proper evaluation of several disease features of high relevance for public health policies.[11]

Our results suggest that serum antibodies from individuals exposed to other recently circulating influenza strains (included in the 2008 and 2009 seasonal influenza vaccines) do not exhibit significant cross-reactivity, as tested by the ELISA method used here.

The present study has a number of limitations. We did not include children under 6 years old because of technical difficulties with schools that manage this young age group. There are no data on baseline seropositivity, which might be important for incidence calculations.[8] Population recruitment was performed by open invitation; therefore, it is difficult to exclude a population bias, as those most interested or with more self-awareness of influenza A/H1N1/2009, or people who had influenza-like symptoms may have had a greater tendency to participate. Finally, the study was also carried out at the end of the second wave of influenza, so the seroprevalence in all risk groups could change, since the sampling interval may not have been long enough for antibody generation in some of the study participants.

The major contribution of this study is that it makes a direct estimation of the post-wave seropositivity to influenza A/H1N1/2009 virus in the metropolitan area according to the potential of transmission risk and distribution of the groups. Students might be considered as a group for vaccination to a higher extent.

In conclusion, this serological study shows the true extent of influenza A/H1N1/2009 infection in Monterrey, Mexico in 2009 for the selected risk groups, and has provided valuable insights into the epidemiology of the disease by potential transmission risk groups, especially that of students. Although we have to consider the fact that there was no baseline prevalence with which to compare the post-pandemic prevalence across the different age groups, and the limited data on the sensitivity and specificity of the ELISA method by age,

these findings should be applicable to other countries that have experienced a similar pattern of infection. Continued studies to assess changes in the population over time will further improve our understanding of the transmission of influenza A/H1N1/2009, particularly the role of children and adolescents in transmission, and will also provide more robust data regarding disease burden, intervention strategies, and future prevention policies.

Acknowledgments

We wish to thank Mónica Serrano MD, Juan Robledo MD, Abel Barragán MD, Carlos Cuello MD, and Jesús Santos MD, for their technical and logistic support, the team of nurses, and the team of pollsters for their collaboration during the fieldwork. We appreciate the invaluable collaboration of the following institutions: Instituto Tecnológico y de Estudios Superiores de Monterrey-Metropolitan area of Monterrey, Hospital San José Tec de Monterrey, Clínica Nova, Clínica Cuauhtémoc y Famosa, American Institute of Monterrey, Euroamerican School, all in-resident adult homes, and the rest of the participant schools for their logistic support.

The project was funded by the Zambrano-Hellion family, FEMSA, the Biotechnology and Health School, School of Medicine, Instituto Tecnológico y de Estudios Superiores de Monterrey (seed fund CAT-122). We also received support from the National Institutes of Health, National Institute of Allergy and Infectious Diseases, under Contract No. HHSN266200700005C, Cancer Center core grant CA21765, the American Lebanese Syrian Associated Charities (ALSAC), and the Children's Infection Defense Center (CIDC) at St. Jude Children's Research Hospital. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

We acknowledge with thanks the financial support and unconditional trust of the Zambrano-Hellion family, FEMSA, and Tecnológico de Monterrey. We thank the Mexican Government, in particular Dr José Ángel Córdoba Villalobos, Minister of Health in Mexico, for his valuable support, and the State education authorities. No compensation was received for any of the contributions.

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Table 1

Seroprevalence to influenza A/H1N1/2009 virus by potential risk group of transmission

Potential risk group of transmission	Sample proportion, *n* (%)	Age, mean ± SD	Seropositive subjects	Seropositivity prevalence, % (95% CI)[a]
Total sample	2222 (100%)	32.6 (21.4)	859	38.7% (36.7–40.7)
Risk group[b]				
Student[c]	994 (45%)	14.9 (5.1)	470	47.3% (44.2–50.4)
Teacher[d]	360 (16%)	42.0 (10.3)	122	33.9% (29.2–38.9)
Healthcare worker[e]	309 (14%)	38.1 (10.0)	76	24.6% (20.1–29.7)
Adult >60 years[f]	189 (9%)	82.3 (9.1)	69	36.5% (30.0–43.6)
General population[g]	370 (17%)	40.6 (11.9)	122	33.0% (28.4–37.9)

SD, standard deviation; CI, confidence interval.

[a] Seropositivity prevalence data represent percentage and 95% CI of individuals with positive antibodies in that group.

[b] Risk group: groups according to potential risk of transmission of influenza A/H1N1/2009 virus; *p*-value = 0.011 obtained from a logistic regression model (backward, stepwise) adjusting for gender, age, and group.

[c] Student: from elementary school to college students.

[d] Teacher: from elementary school to college teachers in contact with students for at least 6 h/day.

[e] Healthcare worker: Doctors, nurses, lab personnel, and technicians in contact with patients for more than 8 h/day.

[f] Adults >60 years: institutional home residents.

[g] General population: adults aged 20–60 years not included in the other groups and not pregnant.

Table 2

Seroprevalence to influenza A/H1N1/2009 virus by school level

School level potential risk group of transmission	Sample proportion, *n* (%)	Age, mean ± SD	Seropositive subjects	Seropositivity prevalence, % (95% CI)[a]
Student[b]	994 (100%)	14.9 (5.1)	470	47.3% (44.2–50.4)
Elementary	391 (39%)	9.8 (2.4)	206	52.7% (47.7–57.6)
Middle school	76 (8%)	13.5 (1.1)	43	56.6% (45.4–67.1)
High school	225 (23%)	16.3 (1.0)	127	56.4% (49.9–62.8)
College	302 (30%)	20.9 (2.4)	94	31.1% (26.2–36.6)
Teacher[c]	360 (100%)	42.0 (10.3)	122	33.9% (29.2–38.9)
Elementary	114 (32%)	39.0 (10.5)	34	29.8% (22.2–38.8)
Middle school	19 (5%)	46.9 (10.4)	6	31.6% (15.4–54.0)
High school	79 (22%)	39.8 (9.5)	16	20.3% (12.9–30.4)
College	148 (41%)	44.9 (9.5)	66	44.6% (36.8–52.6)

SD, standard deviation; CI, confidence interval.

[a] Seropositivity prevalence data represent percentage and 95% CI of individuals with positive antibodies in that group.

[b] Student: p-value < 0.001.

[c] Teacher: p-value = 0.002.

p-Values were obtained from a Chi-square test. Logistic models (backward, stepwise) showed gender and age not to be significant for both the student and teacher groups.

Table 3

Seroprevalence to influenza A/H1N1/2009 virus by gender and age grouped into decades

Characteristic	Sample proportion, *n* (%)	Age, mean ± SD	Seropositive subjects	Seropositivity prevalence, % (95% CI)[a]
Gender[b]				
Male	870 (39%)	28.4 (19.8)	364	41.8% (38.6–45.1)
Female	1352 (61%)	35.2 (22.0)	495	36.6% (34.1–39.2)
Age grouped into decades[c]				
6–10	229 (11%)	8.2 (1.5)	117	51.1% (44.7–57.5)
11–20	623 (29%)	15.8 (2.8)	305	49.0% (45.0–52.9)
21–30	321 (15%)	24.6 (3.0)	119	37.1% (32.0–42.5)
31–40	311 (14%)	35.7 (2.9)	84	27.0% (22.4–32.2)
41–50	313 (14%)	45.3 (2.9)	91	29.1% (24.3–34.3)
51–60	143 (7%)	54.8 (2.9)	36	25.2% (18.8–32.9)
61–70	45 (2%)	64.5 (2.7)	16	35.6% (23.2–50.2)
71–80	61 (3%)	76.2 (2.8)	25	41.0% (29.5–53.5)
81–90	80 (4%)	85.5 (2.5)	30	37.5% (27.7–48.5)
91+	34 (2%)	94.5 (3.4)	13	38.2% (23.9–55.0)

SD, standard deviation; CI, confidence interval.

[a] Seropositivity prevalence data represent percentage and 95% CI of individuals with positive antibodies in that group.

[b] Gender: p-value = 0.259.

[c] Age cohort: p-value < 0.000; 62 missing values for decade of birth.

p-Values were obtained from a logistic model adjusted for gender and age.