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Pandemic influenza A/H1N1 2009 antibodies in the metropolitan area of Buenos Aires in Argentina☆☆

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SUMMARY

Objective: To estimate the infection prevalence in Buenos Aires during the outbreak of pandemic influenza A/H1N1 2009 virus (A(H1N1)pdm09).
Methods: A(H1N1)pdm09-specific antibodies were measured by hemagglutination inhibition assay in human serum samples collected 6 months after the outbreak and before the introduction of the A(H1N1)pdm09 vaccine in Argentina. Baseline levels of cross-reactive antibodies to A(H1N1)pdm09 were determined by testing 162 serum samples collected before 2009.
Results: The overall seroprevalence of A(H1N1)pdm09 in 150 children and 427 adults was 28.9% (95% confidence interval (CI) 25–33%), with a 58.0% prevalence in children <19 years of age and an 18.7% prevalence in adults ≥19 years of age ($p < 0.001$). The prevalence was 43.5% in children <5 years old and 60.6% among children aged 5–18 years. The prevalence in adults declined with increasing age: 24.9% in 19–39-year-olds, 9.7% in 40–59-year-olds, and 8.1% in those ≥60 years old. The prevalence of specific A(H1N1)pdm09 antibodies was higher compared with the baseline in children ($p = 0.014$), adolescents ($p < 0.001$), and adults <40 years old ($p = 0.017$). Seroprevalence in health care workers was not different from the rest of the population (13.6% vs. 19.3%, respectively; $p = 0.421$).
Conclusions: The prevalence of specific A(H1N1)pdm09 antibodies was high at 28.9%. The highest prevalence was observed in children, adolescents, and young adults.

1. Introduction

At the end of March 2009, during the early spring, an outbreak of illness caused by a novel swine-origin influenza A/H1N1 virus was identified in Mexico;[1] this constituted the first wave of the outbreak that was less severe than the second wave in the Northern Hemisphere.[2,3] In June 2009, the World Health Organization (WHO) declared that the rapidly spreading swine-origin influenza A/H1N1 virus constituted a global pandemic.[4] Many countries made detailed plans to mitigate the clinical and societal effects of the pandemic. The A(H1N1)pdm09 virus caused over 277 607 laboratory-confirmed cases and over 3205 deaths worldwide as of September 6, 2009,[5] but national and international authorities have acknowledged that these counts are substantial underestimates, reflecting an inability to identify, test, confirm, and report many cases, especially mild cases.[6] Recently, Dawood et al. estimated 300 000 deaths during the first year of A(H1N1)pdm09 circulation.[7] Miller et al. reported the results of a large seroepidemiological survey in England to document the age-specific prevalence of neutralizing antibodies to A(H1N1)pdm09 before and after the first wave of the pandemic, to provide a direct measure of the incidence of infection; they concluded that it was approximately 2% at baseline and between 20% and 40% for different age groups among children, but was not different in adults.[8] However, the impact of influenza during the spring and

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summer of 2010 in the Northern Hemisphere was lower than that observed during the first wave in the Southern Hemisphere.[9]

The first outbreak of A(H1N1)pdm09 reached the metropolitan area of Buenos Aires, inhabited by 14 million people, in May 2009, and led to the highest mortality rates among patients confirmed to be infected with this virus in the Southern Hemisphere.[10]

This report describes a cross-sectional serological study from one of the countries in the Southern Hemisphere where the first wave of circulation of the A(H1N1)pdm09 virus coincided with seasonal influenza infections. Virus-specific antibody levels were measured in community participants and health care workers (HCWs) after this first wave of infection. The prevalence was estimated by measuring neutralizing antibodies to the A(H1N1)pdm09 virus using pre-pandemic (baseline) and post-pandemic serum samples.

2. Materials and methods

This study was performed during January and February 2010 (the summer season in Argentina) after the main wave of A(H1N1)pdm09 infection had occurred in the country and before the nationwide vaccination campaign for the A(H1N1)pdm09 virus started in March 2010 (Figure 1).

This was a hospital-based population study. Two public hospitals were involved in the study: Hospital de Niños "Dr. Ricardo Gutiérrez", a pediatric hospital associated with the Universidad de Buenos Aires, and Hospital de Clínicas "José de San Martín", Universidad de Buenos Aires. The hospitals serve as reference centers for pediatric and adult patient populations, respectively.

2.1. Study population

The study population included otherwise healthy children <19 years old undergoing elective surgery who required routine laboratory analyses at Hospital de Niños "Dr. Ricardo Gutiérrez", and healthy adults and blood donors from Hospital de Clínicas "José de San Martín".

Subjects older than 1 year who agreed to participate in the study and who provided written informed consent were included. For children aged <19 years, written informed consent was provided by their parents or legal representatives.

In addition, blood samples were obtained from 40 healthy HCWs, including physicians, nurses, administrative personnel, and laboratory technicians who worked at the Hospital de Clínicas during the pandemic, to compare the prevalence in this population with the prevalence observed in the rest of the adult population under study.

Subjects diagnosed with AIDS, those receiving chronic corticosteroid therapy (equivalent to meprednisone ≥20 mg/day for at least 1 month), patients with active malignancies under therapy, and organ transplant recipients undergoing immunosuppressive therapy were excluded.

This study was conducted in accordance with the amended Declaration of Helsinki and was approved by the institutional review boards and ethics committees of both participating hospitals (approval numbers 121109 and 161209 for Hospital de Niños "Dr. Ricardo Gutiérrez" and Hospital de Clínicas "José de San Martín", respectively).

2.2. Questionnaire

A questionnaire was completed to collect patient demographic data and information on relevant past medical history, the occurrence of suspected symptoms of influenza during the past fall–winter season (May to October 2009) in the Southern Hemisphere, history of seasonal influenza vaccination, household contact with a suspected or confirmed A(H1N1)pdm09 case, and the use of antiviral and/or antibiotic medication during the influenza season.

2.3. Specimen collection

For both adults and children, a 1.5-ml blood sample was drawn for measurement of antibodies against the A(H1N1)pdm09 virus. Serum samples were obtained by centrifugation for 10 min at 2500 rpm and stored at −20 °C. The analyses to determine the antibody titers were performed by the Virology Department, Instituto Nacional de Enfermedades Infecciosas – ANLIS, "Dr. C.G. Malbrán", Buenos Aires.

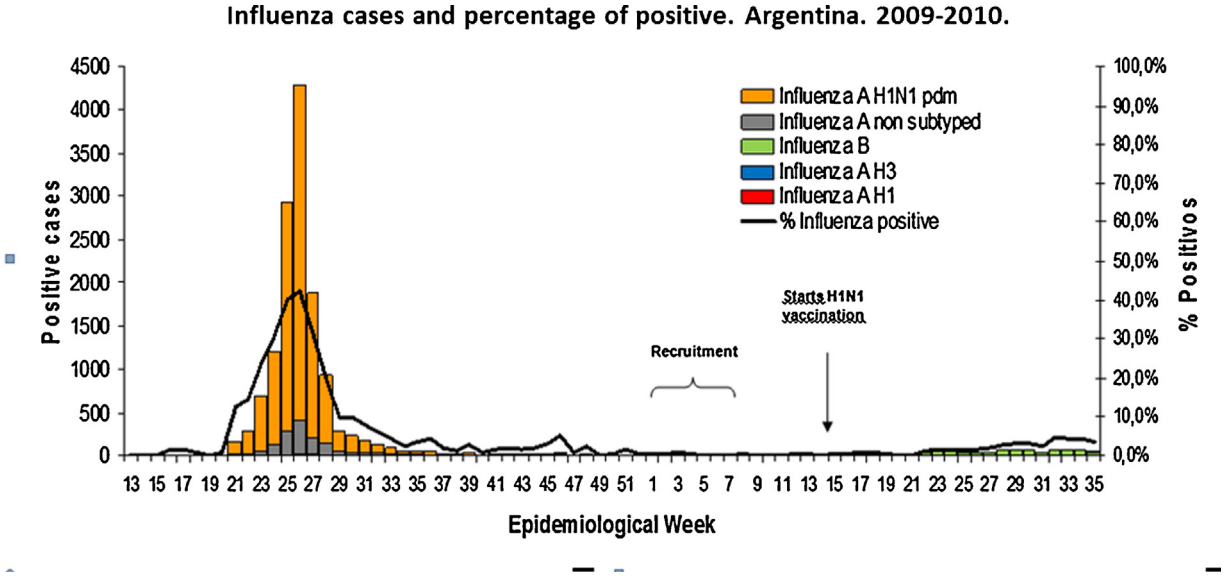

Figure 1. The incidence of influenza in Argentina from week 13, 2009 to week 35, 2010. The A(H1N1)pdm09 outbreak produced a peak of incidence that occurred at week 26 in 2009. In contrast to what happened in 2009, the incidence of A(H1N1)pdm09 in 2010 was very low. Instead, during 2010, the most prevalent influenza virus was influenza B. Reproduced with permission from Sistema de Vigilancia por Laboratorio (SIVILA-SNVS), Dirección de Epidemiología, Ministerio de Salud de la Nación.

2.4. Laboratory methods

Antibodies against the A(H1N1)pdm09 virus were detected using the hemagglutination inhibition (HI) assay. Serum samples were pre-treated with receptor destroying enzyme (RDE; Denka Seiken Co. Ltd, Tokyo, Japan) at a 1:4 (vol/vol) dilution and incubated overnight at 37 °C. The enzyme was inactivated by heating at 56 °C for 30 min. The HI assay was performed according to the standard protocol provided by the WHO Collaborating Centre for Reference and Research on Influenza and the US Centers for Disease Control and Prevention (CDC, Atlanta, USA).

Briefly, serum samples were titrated in phosphate buffered saline using two-fold dilutions from 1:10 to 1:1280 and incubated with 25 μl (4 hemagglutinating units (HAU)) of β-propiolactone (BPL)-inactivated A(H1N1)pdm09 antigen provided by the CDC. Following 1 h of incubation, 25 μl of a 0.5% turkey red blood cell suspension were added to each well.

When the serum sample presented non-specific agglutinins, 20 volumes of the serum were adsorbed with one volume of packed turkey red blood cells. An A(H1N1)pdm09 reference sheep antiserum provided with the CDC kit was included as a positive control, and an influenza-negative sheep serum was used as a negative control. The HI titer is the reciprocal of the last dilution of antiserum that completely inhibits hemagglutination. It is generally accepted that serum HI antibody titers of 40 are associated with at least a 50% reduction in risk for infection or disease from influenza viruses in human populations.[11] Therefore, an HI antibody titer ≥40 was considered a positive result.

2.5. Control population

A baseline level of prevalence of cross-reactive antibodies to the A(H1N1)pdm09 virus before the virus was introduced into the country was determined using serum samples collected as part of a seroepidemiological study of viral hepatitis during 2007–2008 from individuals aged 1–73 years living in the same region.

2.6. Data analysis and statistics

The estimated sample size for a confidence level of 95% was 384 participants, assuming a prevalence of 50%, a precision level of 5%, and a half interval of 20%.

A descriptive analysis was performed. For categorical variables, the Chi-square or Fisher's exact test was used. For numerical data, the Mann–Whitney test was performed. A p-value of <0.05 was considered statistically significant. The statistical package SPSS Statistics version 17.0 (SPSS Inc., Chicago, IL, USA) was used for the analyses.

Table 1
Characteristics of the study population

	Children (n = 150)	Adults (n = 427)
Age[a]		
Mean (± SD)	130.3 (± 57.7)	38.5 (± 14)
Median	136.5	35.5
Range	18–216	19–80
Gender		
Female	85 (56.7%)	198 (46.6%)
Location		
CABA	31 (20.7%)	161 (37.8%)
Suburban area	114 (76.0%)	260 (60.8%)
Interior regions of the country	5 (3.3%)	5 (1.2%)
Health care workers	-	44 (10.3%)

SD, standard deviation; CABA, Ciudad Autónoma de Buenos Aires.
[a] Age is expressed in months for children and in years for adults.

3. Results

Serum samples collected from 577 people aged 18 months to 80 years were included in the study (Table 1). Overall, 28.9% (95% confidence interval (CI) 25–33%) of the study population had seroprotective HI antibody levels to A(H1N1)pdm09 in January and February 2010.

The proportion of people with seroprotection against the A(H1N1)pdm09 virus varied with age (Figure 2). The seroprevalence was 58.0% (95% CI 49.7–66.2%) in children aged 1–18 years and 18.7% (95% CI 14.9–22.5%) in adults ($p < 0.001$).

In children, seroprevalence increased according to age. From 12 to 59 months, 10/23 were seropositive (43.55%, 95% CI 21–65.9%); from 60 to 119 months, 25/40 were seropositive (62.5%, 95% CI 46–78%); from 120 to 179 months, 33/49 were seropositive (67.3%, 95% CI 53–81%); and from 180 to 216 months, 19/38 were seropositive (50%, 95% CI 32–67%).

School-aged children, 5–18 years old, had the highest seroprevalence and a significant increase compared to the baseline ($p < 0.001$). This was followed by pre-school children aged 1–4 years ($p = 0.014$).

Seroprevalence was lower among adults aged 19–39 years, with a significant increase compared to the baseline ($p = 0.017$). It was even lower among those aged 40–59 years and among those aged ≥60 years. For the age groups 40–59 years and ≥60 years, seroprevalence was not significantly higher than the prevalence found at baseline.

Data obtained using the questionnaire (Table 2) indicated that in children, there was no statistically significant difference in seroprevalence by sex or location; however, in adults, men had a significantly higher seroprevalence than women. The seroprevalence among HCWs was not higher than the seroprevalence found in the rest of the sampled adult population.

All of the enrolled subjects were asked about the occurrence of symptoms of influenza-like illness during the fall–winter 2009 season. Recorded symptoms included a history of fever, sore throat or hoarseness, nasal discharge or bleeding, dry or productive cough, shortness of breath, intense muscle or joint pain, nausea or vomiting, diarrhea, headache, and conjunctivitis. Only headache was significantly associated with seroprotection in both children ($p = 0.016$) and adults ($p = 0.020$).

Interestingly, among children, a history of nausea or vomiting showed a significant inverse association with the presence of protective titers of antibodies against A(H1N1)pdm09 ($p = 0.040$).

In children, no relevant past medical history was identified as a risk factor for A(H1N1)pdm09 infection, including past neoplastic disease, diabetes, asthma, or prematurity. None of the seropositive children had possible or confirmed exposure to this virus.

Among adults, current cigarette smoking was associated with acquisition of the infection ($p = 0.034$). Past neoplastic disease, diabetes, HIV positivity, any heart or lung disease, pregnancy during 2009, and heavy drinking were not significantly associated with seroprotection. Among subjects who received oseltamivir treatment during that influenza season, 75% had detectable A(H1N1)pdm09 antibody titers ($p = 0.006$).

4. Discussion

In this study, we observed that overall seroprevalence of antibodies against the A(H1N1)pdm09 virus for the population attending one of two hospitals in the metropolitan area of Buenos Aires was 29%. There was wide variation in the acquired seroprotection level against the A(H1N1)pdm09 virus among the different age groups. Seroprevalence in subjects aged 1–18 years was 58.0% (60% in school-aged children, 5–18 years), while it was 18.7% in adults. Although this difference has been mentioned

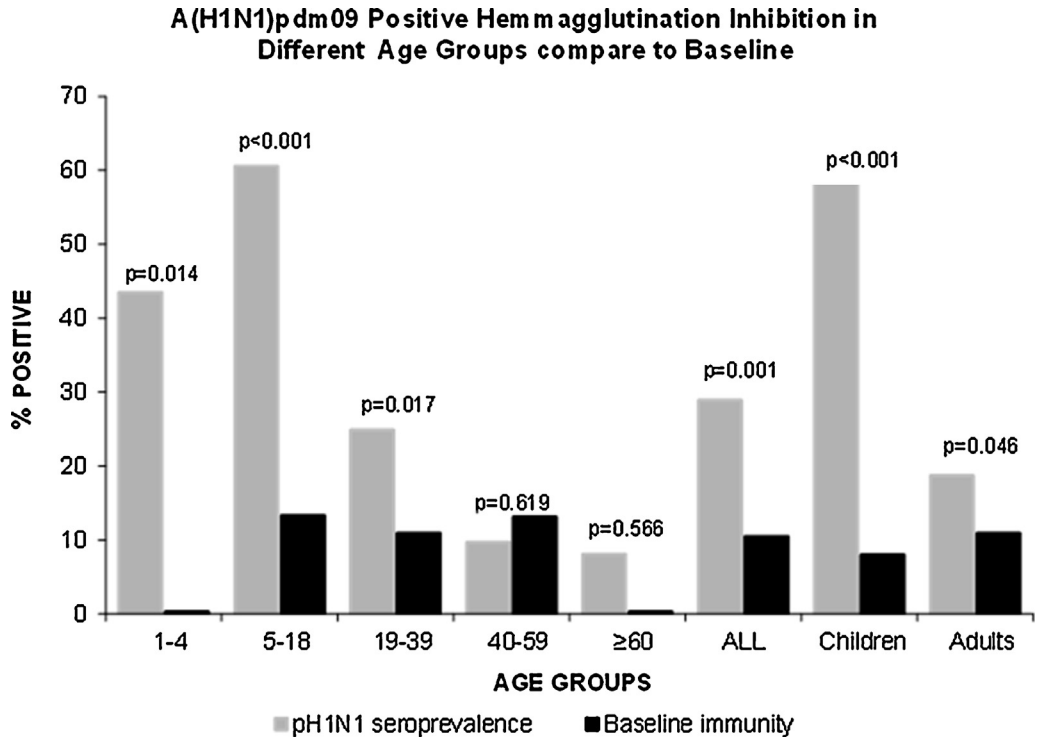

Figure 2. The seroprevalence of A(H1N1)pdm09 and its variation among the different age groups compared with the baseline titers. Seroprevalence was 58% (95% CI 49.7–66.2%) in children 1–18 years old and 18.7% (95% CI 14.9–22.5%) in adults ($p < 0.001$). Among children, the seroprevalence was 43.5% (95% CI 21–65.9%) in those aged 1–4 years and 60.6% (95% CI 51.7–69.5%) in school-aged children (5–18 years old); in both groups, the seroprevalence increased significantly compared to the baseline. Among adults 19–39 years of age, the seroprevalence was 24.9% (95% CI 19.4–30.4%), which was significantly higher than the baseline. Among those aged 40–59 years old, the seroprevalence was 9.7% (95% CI 4.3–15.1%), and among those aged ≥60 years, it was 8.1% (95% CI 0–18.3%). For these two groups, seroprevalence did not increase significantly compared to the baseline.

in other studies describing the epidemiology of the 2009 pandemic, the prevalence found in this study is higher than the figures reported previously.[8,12] In a cross-sectional serological survey performed in England, Miller et al. compared the prevalence observed in serum samples taken in 2008 (before the first wave of A(H1N1)pdm09 infection) with serum samples taken in August and September 2009 (after the first wave of infection); they observed a significant increase in the HI titers for subjects between 0 and 24 years of age, but not in the older age groups.[8] The prevalence of positive HI serology they reported was about half that reported here. This difference could be attributed to the relatively mild characteristics of the first wave of infection that occurred in the spring season in the Northern Hemisphere, while a more severe wave occurred during the fall and winter seasons in the Southern Hemisphere.[6] Higher attack rates during the first wave were reported in Australia, New Zealand, South Africa, and South America.[10] Bandaranayake et al. in New Zealand measured the titers of neutralizing antibodies after the first wave of A(H1N1)pdm09 and compared pre-pandemic with post-pandemic seroprevalence in non-vaccinated subjects in the

Table 2
Pandemic influenza A/H1N1 2009 seroprevalence by gender, location, and health care worker status

	n	Positive titer ≥40	Seroprevalence % (95% CI)	*p*-Value for group
Gender				
Children				1
Female	85	49	57.6 (46.5–68.7)	
Male	65	38	58.5 (45.7–71.2)	
Adults				0.013
Female	198	27	13.6 (8.6–18.4)	
Male	229	53	23.2 (17.4–28.8)	
Location				
Children				0.690
CABA	31	16	51.6 (32.4–70.8)	
Suburban area	114	69	60.5 (51.1–69.9)	
Inner of the country	5	2	40.0 (0–92.9)	
Adults				0.552
CABA	161	26	16.1 (10.1–22.1)	
Suburban area	260	53	20.3 (15.2–25.4)	
Inner of the country	5	1	20 (0–65.0)	
Health care workers				0.421
Yes	44	6	13.6 (2.3–24.9)	
No	383	74	19.3 (15.2–23.4)	

CI, confidence interval; CABA, Ciudad Autónoma de Buenos Aires.

general population and in HCWs.[12] Similar to our findings, they found an overall community seroprevalence of 26.7%; analyzing the different age groups, they described a seroprevalence of 46.7% in children aged 5–19 years, with a significant increase from the baseline, while in older adults aged ≥60 years, seroprevalence was not different compared with the baseline. They also found, as we did, that seroprevalence in HCWs was not different from that observed in the general population. General hygiene measures strictly established during the pandemic, as well as the use of post-exposure prophylaxis, could explain this result. The lower seroprevalence observed in children aged 5–19 years in the New Zealand study compared with children aged 5–18 years in our study suggests that a stronger impact of infection existed in Argentina than in New Zealand.

In Argentina, Libster et al. performed a study of the burden of the disease caused by the A(H1N1)pdm09 virus during an expected second wave in a catchment population of 1.2 million children. While there were 251 hospitalizations and 13 deaths between May 1 and July 31, 2009, no pediatric hospitalizations due to A(H1N1)pdm09 were identified in 2010 ($p < 0.001$).[13] They attributed this absence of severe pediatric cases mainly to the administration of the A(H1N1)pdm09 monovalent vaccine in infants, children under 5 years of age, and older children with high-risk medical conditions, as well as to the availability and use of oseltamivir and to the effect of the 2009 outbreak leaving natural immune protection. A similar study performed in New Zealand in 2010 showed that there was a less severe impact of A(H1N1)pdm09 in 2010 than that observed in 2009.[14] Because the vaccination campaigns in both countries during the first months of 2010 were similar, with coverage of approximately 25% of the population,[14,15] the difference in attack rates is probably more attributable to the higher attack rate observed in Argentina during the winter in 2009, particularly in school-aged children.

In our study, the seroprevalence was lower in adults, although it remained significantly higher than the baseline in people aged 19–39 years. The seroprevalence was even lower among those aged 40–59 years and in those aged ≥60 years. For these last two groups, seroprevalence was not significantly higher than the prevalence found at baseline.

Since 1981, the CDC has recommended that HCWs receive vaccination against influenza in an effort to reduce transmission of the virus to their vulnerable patients.[16] It has been documented that nurses from a staff cohort with a vaccination rate near null were the likely source of devastating influenza outbreaks in a neonatal unit[17] and in a solid-transplant unit.[18] In 2009, during the A(H1N1)pdm09 pandemic, immunization was recommended to HCWs, regardless of whether or not they had vulnerable patients in their care, to protect themselves, their families, and their patients from influenza. Comparing the seroprevalence of A(H1N1)pdm09 found among the HCWs with the general population, neither our study nor the one by Bandaranayake et al. found a higher seroprevalence in HCWs.[12] These findings may reflect that transmission of A(H1N1)pdm09 occurred mainly in the community, with a reduced transmission rate among hospital admissions.

We found a significantly higher seroprevalence in male adults but not in male children. This may be due to a higher exposure rate in adult males than in females because of mitigation measures implemented during the pandemic, such as closing schools, duty leave for pregnant women, and other guidelines that mainly reduced the exposure of women.

Headache, both in children and in adults, was the only clinical manifestation that was recalled by the seropositive individuals as being present during the time of the outbreak in Buenos Aires, in contrast with the usual flu symptoms of high fever, respiratory signs, and diarrhea in children. Interestingly, among children, a history of nausea or vomiting showed a significant inverse association with the presence of protective titers of antibodies against A(H1N1)pdm09, probably reflecting a gastrointestinal illness rather than an influenza-like symptom.

In a case–control study of risk factors for hospitalization caused by A(H1N1)pdm09, Ward et al. found smoking to be an independent risk factor for hospitalization from this virus,[19] which is consistent with our findings of current smoking being associated with the acquisition of this infection. In addition, Muscatello et al. also found smoking to be an independent risk factor associated with influenza-like illness during the 2009 winter epidemic of the A(H1N1)pdm09 virus in New South Wales, Australia.[20]

The seroprevalence observed in the four subjects with reported ingestion of oseltamivir during the time of the A(H1N1)pdm09 outbreak in Buenos Aires was 75%, which was significantly higher than in those who did not receive this antiviral therapy. The relative lack of association with the majority of signs, symptoms, and medical history expected to be found in a population with serological positivity for influenza suggests that the spread of the A(H1N1)pdm09 infection was often subclinical and could not be recognized by those infected.

The present study has some limitations. While blood donors contributed to the pool of sampled adults, the sampled population of children only included those who were otherwise healthy and undergoing elective surgery requiring blood tests. In addition, we cannot exclude the possibility that the relatively small proportion of HCWs in this study makes the comparison of their seropositivity with that of the rest of the population less precise.

In conclusion, the seroprevalence of the A(H1N1)pdm09 virus in the metropolitan area of Buenos Aires was high, likely due to several factors, including the appearance of the infection during the winter in the Southern Hemisphere and its simultaneous introduction into different settings at the same time. The seroprevalence was much higher in children, who constitute the main age group responsible for spreading the infection due to their having no pre-existing immunity against this novel virus. Interestingly, headache was the only reported symptom by those having the infection, and seroprevalence in HCWs was not higher than that in the general population.

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Ethical approval: This study was conducted in accordance with the amended Declaration of Helsinki and was approved by the institutional review boards and ethics committees of both participating hospitals (approval numbers 121109 and 161209 for Hospital de Niños "Dr. Ricardo Gutiérrez" and Hospital de Clínicas "José de San Martín", respectively).

Conflict of interest: Carlos M. Luna has been a consultant or lecturer for Pfizer, Bayer, Merck Sharp and Dohme, and AstraZeneca, and has received research support from Pfizer. No conflicts exist for the remaining co-authors.

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