Clinical and Epidemiological Features of 2009 Pandemic H1N1 Influenza Differ Slightly According to Seroprevalence Status During the Second Wave in the General Population in México

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BACKGROUND: Clinical features of pandemic H1N1 have been derived from lab-confirmed, hospitalized, or critically ill subjects. This report describes the clinical features of H1N1 and their prevalence from non-confirmed subjects according to seroprevalence status in México. The objective was to determine the prevalence of these clinical features from non-confirmed cases of pandemic H1N1 and to compare them according to seroprevalence status in northern Monterrey, México, during 2009, and to identify the predictive signs and symptoms; there have been no prior serologic studies in México. METHODS: During November–December 2009, 2,222 volunteers, ages 6–99 years, were categorized into 3 symptomatic groups: influenza-like illness, respiratory illness, and non-respiratory illness. Antibodies against influenza A/H1N1/2009 were determined by a virus-free enzyme-linked immunosorbent assay (ELISA) method. Demographics and clinical presentation were assessed through face-to-face questionnaire, and the association with seroprevalence status was determined and compared. RESULTS: Overall seroprevalence was 39%. Of the seropositive subjects, 67% were symptomatic and 33% were asymptomatic. Seventy-one percent of seropositive symptomatic subjects reported respiratory illness, 17% reported non-respiratory symptoms, and 12% reported influenza-like illness. The most common symptoms were rhinorrhea/nasal congestion (93%) and headache (83%). No significant difference was found between the symptom profiles of the seropositive group, compared to the seronegative one, nor of the median duration of symptoms. The seropositive group had a significantly elevated proportion of influenza-like illness (12%), compared to the seronegative group (8%). The proportion of subjects who took days off and who sought medical attention was significantly higher in the seropositive group. No single symptom was associated as a predictor of seropositiveness. CONCLUSIONS: One third of the seropositive subjects were asymptomatic, and few had an influenza-like illness. No difference was found in the symptom profiles of the seropositive and seronegative groups. No single symptom predicted seropositiveness. Large scale population studies are needed, especially in México, to characterize clinical syndromes. *Key words: clinical features; 2009 pandemic H1N1; seroprevalence; México; seropositive status; serologic; H1N1 symptoms.* [Respir Care 2012;57(10):1586–1593. © 2012 Daedalus Enterprises]

Introduction

In April 2009, a novel human infection with the 2009 pandemic influenza A (H1N1) virus emerged in the United States[1] and México,[2] and then spread globally.[3–5] A second wave swept through México, peaking by early October and returning to baseline levels by early December, 2009.[6]

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Most illnesses caused by the 2009 H1N1 virus have been acute and self-limited, with the highest attack rates reported among children and young adults. Most of the serious illnesses have also occurred among children and nonelderly adults, with approximately 90% of deaths occurring in those under 65 years of age.[7] The relative sparing of adults older than 60 years of age[8,9] is presumably due to their exposure to antigenically related influenza viruses earlier in life, resulting in the development of cross-protective antibodies.[7,10] Infection with the 2009 H1N1 virus causes a broad spectrum of clinical syndromes, ranging from afebrile upper respiratory illness to fulminant viral pneumonia.[7,8,11] The clinical diagnosis of influenza infection is, however, often elusive, given its non-specific presentation.[12] The use of a simple symptom complex for influenza-like illness (ILI) at the primary care level can serve as a convenient predictive tool for influenza infection, especially in the setting of an influenza community outbreak. However, the sensitivity and positive predictive value of such symptom complexes or definitions vary widely, depending on the prevalence of the disease and population tested.[12,13]

In the face of an influenza pandemic, accurate estimates of epidemiologic parameters are required to help guide decision-making. Most of the clinical features of influenza A/H1N1/2009 cases have been determined in studies addressing lab-confirmed, hospitalized or critically ill subjects, but not from an assessment of the clinical features of non-confirmed cases from the general population. We undertook this study: to ascertain the seroprevalence of H1N1 in non-confirmed cases from the general population in northeastern México; to estimate the epidemiological features as well as the clinical presentation for both, seropositive (positive antibodies to influenza A/H1N1/2009 virus) and seronegative subjects from the general population in the community setting; and to determine the predictive symptoms according to seroprevalence results.

QUICK LOOK

Current knowledge

Reports of the clinical features of H1N1 in patients with less severe flu symptoms are not well described, and there is no method to identify predictive signs in this group.

What this paper contributes to our knowledge

The seroprevalence of influenza A/H1N1/2009 in Monterrey, northern Mexico, was high. The majority of those infected had mild or no illness, and the clinical presentation was highly variable. No single symptom was a predictor of seropositive response.

Methods

Subjects

This was a cross-sectional study of 2,222 subjects, ages 6–99 years, whose serum samples were collected between November 9 and December 17, 2009, in the metropolitan area of Monterrey, in northeastern México. The region has a population of 2,708,529 persons.[14] Subjects were divided into 2 groups: symptomatic and asymptomatic subjects. Symptomatic subjects were categorized in age cohorts and in 3 mutually exclusive symptom profile groups: ILI, respiratory illness, and non-respiratory illness. Table 1 shows the number and proportion of the subjects for each age cohort. An open invitation to participate voluntarily in the study was made to the community through letters, telephone calls, and flyers. Blood samples were drawn on site in 6 elementary and middle schools, 4 high schools, one university, 4 in-resident older adult homes, and 3 hospitals. Samples from the general population group, from all over the metropolitan area, were collected at one single site, the School of Medicine Lab-Tecnológico de Monterrey. Geographical localization of schools, older adult homes, hospitals, and people from the general population group, as well as the proportion of people from each decade of birth in the metropolitan area, make the study population representative of the metropolitan area (with exception of children < 5 y). Inclusion criteria were voluntary participation and overnight fasting. The only exclusion criterion was age younger than 5 years. Written informed consent was obtained from all subjects and from parents of those younger than 18 years. Approvals by the ethics and research committees of the School of Medicine Tecnológico de Monterrey and by the Education and Health Secretariats were obtained.

Trained pollsters administered a questionnaire to each subject. Parents answered the questionnaire in cases of children and adolescents younger than 15 years old. The questionnaire included the following data: demographics, symptoms (fever, defined as a body temperature > 37.8°C, cough, rhinorrhea, nasal congestion, myalgia, arthralgia, headache, eye pain, diarrhea, vomiting, and abdominal pain), date of onset, duration of symptoms, number of days

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Table 1. Demographic Characteristics of Subjects

	Total (N = 2,222)		
	n	%	95% CI
Male	870	39	37–41
Female	1,352	61	59–63
Age Group, y			
6–10	229	10	9–12
11–20	623	28	26–30
21–30	321	15	13–16
31–40	311	14	13–15
41–50	313	14	13–16
51–60	143	6	5–7
61–70	45	2	1–3
71+	175	8	7–9
Non-specified	62	3	2–3

off, and vaccination against seasonal influenza A 2008, 2009 or pandemic influenza A/H1N1/2009.

Definitions

We categorized symptomatic subjects into 3 mutually exclusive symptom profile groups: ILI, respiratory illness, and exclusively non-respiratory illness. We defined non-respiratory illness as the presence of headache, abdominal pain, eye pain, myalgia/arthralgia, fever, vomiting and/or diarrhea. Respiratory illness was defined either as the presence of cough or rhinorrhea/nasal congestion or by the presence of temperature $\geq$ 37.8°C, myalgia/arthralgia, or eye pain, plus cough or rhinorrhea/nasal congestion (but without both, fever and cough) on one or more days. Other studies have used the presence of 2 of these signs or symptoms.[15,16] We also used the surveillance definition for ILI (temperature $\geq$ 37.8°C plus cough), as recommended by the Centers for Disease Control and Prevention.[17]

Measurement of Antibodies to Influenza A/H1N1/2009

Antibodies against the pandemic A/H1N1/2009 virus were detected by using a virus-free enzyme-linked immunosorbent assay (ELISA) method. Overnight fasting blood samples were drawn from subjects, centrifuged within 3 hours, and frozen at −80°C. The virus-free ELISA method,[18,19] 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, Oxford, United Kingdom) in PBS (phosphate-buffered saline) was dispensed into microassay plate wells (Maxisorp, Corning, Acton, Massachusetts), incubated, and then repeatedly washed. A blocking buffer (Superblock T20 PBS, catalog no. 37516, Pierce Biotechnology, Rockford, Illinois) was added to block the surface not covered with antibodies, then the wells were washed again. A non-glycosylated, histidine-tagged recombinant protein fragment of the hemagglutinin of influenza A/H1N1/2009 virus, expressed in *Escherichia coli,*[20] was then added. The proper folding of this protein was demonstrated by x-ray crystallography, according to DuBois et al.[21] 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 immunoglobulin G (IgG) antibody solution (1:30000 dilution in PBS-Tween 0.05%) marked with horseradish peroxidase (Pierce Biotechnology, Rockford, Illinois) was used. After incubating and washing, 100 μL/well of substrate solution (1 Step Ultra TMB-ELISA, lot 34028, Pierce Biotechnology, Rockford, Illinois) was added. After incubation, the enzymatic reaction was stopped by adding 50 μL/well of 1 M H_2SO_4. Color produced by the enzymatic reaction was evaluated by absorbance at 450 nm with a microplate reader (BioTek Instruments, Winooski, Vermont). Absorbance values were normalized for each plate, based on the signal of sera 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 $\pm$ 0.25 (mean $\pm$ 1 standard deviation).[18]

From the 2,222 volunteers studied, 950 subjects (mean age 40.8 y) were 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 HI (hemagglutination inhibition) assays, an additional set of 20 serum samples from polymerase-chain-reaction 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. These positive volunteers were recruited from regular patients at Hospital San José Tecnológico de Monterrey and Clínica Nova during October and November 2009 to compare diagnostic performance of the ELISA method against standard HI. Data from this population were not included in the statistical analyses. Samples were taken between 2 and 24 weeks after infection. HI assays were conducted at the Department of Infectious Disease at St Jude Children's

Table 2. Epidemiological Features of Seropositive and Seronegative Subjects

	Total (N = 2,222)			Seropositive (n = 859)			Seronegative (n = 1,363)			P*
	n	%	95% CI	n	%	95% CI	n	%	95% CI	
Total sample	2,222	100		859	39	37–41	1,363	61	59–63	< .001
Asymptomatic	807	36	34–38	287	33	30–37	520	38	36–41	.02
Symptomatic	1,415	64	62–66	572	67	63–70	843	62	59–64	.02
Respiratory illness	1,039	73	71–76	407	71	67–75	632	75	72–78	.11
Influenza-like illness	140	10	8–11	70	12	10–15	70	8	6–10	.02
Non-respiratory	236	17	15–19	95	17	14–20	141	17	14–19	.95
Sought medical assistance	812	37	35–39	354	41	38–45	458	34	31–36	< .001
Mean of duration of symptoms (d)†	3.63		3.5–3.7	3.7		3.5–3.8	3.6		3.4–3.7	.31
Subjects who took days off	390	18	16–19	175	20	18–23	215	16	14–18	< .001
Mean of days off†	2.4		2.2–2.5	2.5		2.3–2.8	2.2		2.0–2.5	.11
History of seasonal influenza vaccination	950	42	40–44	333	38	35–42	617	45	42–47	< .001

* Calculated from 2 proportion comparison method, based on normal distribution for seropositive and seronegative groups.
† Mean represents the average with 95% confidence interval and P for mean comparison based on normal distribution.

Table 3. Symptomatic Profile for Seropositive and Seronegative Subjects

	Total n = 1415		Seropositive n = 572		Seronegative n = 843		P*
	n	%	n	%	n	%	
Respiratory illness	1,039	73	407	71	632	75	.11
Cough only	24	2	8	2	16	3	.54
Cough plus 1 symptom†	101	10	39	10	62	10	.90
Cough plus 2 symptoms†	89	9	36	9	53	8	.80
Cough plus ≥ 3 symptoms†	171	16	70	17	101	16	.61
Rhinorrhea only	240	23	87	21	153	24	.29
Rhinorrhea plus 1 symptom‡	174	17	78	19	96	15	.10
Rhinorrhea plus 2 symptoms‡	125	12	51	13	74	12	.69
Rhinorrhea plus ≥ 3 symptoms‡	115	11	38	9	77	12	.14
Influenza-like illness	140	10	70	12	70	8	.02
Fever and cough	140	100	70	100	70	100	
Rhinorrhea/nasal congestion	127	91	65	93	62	89	.38
Headache	123	88	58	83	65	93	.06
Myalgia/arthralgia	106	76	55	79	51	73	.42
Eye pain	72	51	38	54	34	49	.49
Abdominal pain	32	23	20	29	12	17	.10
Diarrhea	29	21	12	17	17	24	.29
Vomiting	24	17	13	19	11	16	.65
Non-respiratory	236	17	95	17	141	17	.95
Headache	170	72	72	76	98	70	.28
Abdominal pain	87	37	26	27	61	43	.01
Eye pain	58	25	17	18	41	29	.04
Myalgia/arthralgia	44	19	14	15	30	21	.19
Diarrhea	44	19	16	17	28	20	.55
Fever	37	16	15	16	22	16	.97
Vomiting	27	11	10	11	17	12	.71

* Calculated from 2 proportion comparison method, based on normal distribution for seropositive and seronegative groups.
† Symptoms: rhinorrhea, headache, myalgia/arthralgia, eye pain, abdominal pain, diarrhea or vomiting.
‡ Symptoms: headache, myalgia/arthralgia, eye pain, abdominal pain, diarrhea or vomiting.

Research Hospital, Memphis, Tennessee, according to standard methodologies.[18]

Statistical Analysis

Analysis was performed with statistics software (SPSS 17, SPSS, Chicago, Illinois) and spreadsheet software (Excel, Microsoft, Redmond, Washington). A P value of .05 or less was considered statistically significant. The 95% confidence intervals for proportions were obtained using a normal distribution (Z test) method.

Prevalence data are presented as the number of individuals with antibodies divided by the number of individuals tested in that category, with the prevalence percentage and its 95% CI in parenthesis. Differences in proportions were evaluated by Z test. Dependence analysis was performed by logistic regression model as an integral analysis concerned with describing the relationship between a response variable (seropositive) and more explanatory variables (symptoms). Differences in means were evaluated by Z test.

Results

Demographic characteristics of the subjects are shown in Table 1. Table 2 reveals that from the 2,222 subjects, 64% were symptomatic, from April to November 2009, while 36% were asymptomatic. From those symptomatic subjects, overall, 17% reported only non-respiratory symptoms, 73% reported a respiratory illness, and 10% reported an ILI.

The overall proportion of patients who tested positive for antibodies to pandemic influenza A/H1N1/2009 virus was 39%. From this seropositive group, 67% were symptomatic and 33% were asymptomatic, with the difference being significant. Also, the proportion of seropositive symptomatic subjects was significantly higher (67%) than that of seronegative symptomatic subjects (62%). Most of the subjects with positive antibodies reported a respiratory illness (71%), 12% reported an ILI, and 17% reported non-respiratory symptoms (see Table 2). There was no significant difference in the symptom profile presentation between the seropositive and seronegative groups, with the exception of abdominal pain and eye pain for those presenting with non-respiratory symptoms. However, the seropositive group had a significantly higher proportion of ILI (12%), compared to the seronegative one (8%) (Table 3).

The individual symptoms most associated with the presence of antibodies against influenza A/H1N1/2009 were cough, rhinorrhea, and headache, while the least association was found for myalgia and eye pain. However, the odds ratio through logistic regression analysis showed a very weak association of any single symptom as a predictor of seropositiveness (Table 4).

Table 4. Predictive Value of Symptoms For Seropositiveness

Symptom	Odds Ratio*	Probability† (%)
Cough	1.18	54
Rhinorrhea	1.17	54
Headache	1.08	52
Diarrhea	1.07	52
Fever	1.02	50
Vomiting	0.98	49
Abdominal	0.95	49
Eye pain	0.94	48
Myalgia	0.85	46

* Odds ratios were calculated with logistic regression analysis.
† Probability calculation was derived from odds ratio.

Regarding other epidemiological features, there was no significant difference for the median duration of symptoms (approximately 4 d) between the seropositive and the seronegative groups (see Table 2). People with more respiratory symptoms showed a linear trend to take longer to recover ($P < .001$). For those who were sick for 3 days or less, only 6.0% presented with ILI, but for those whose symptoms lasted 4 or more days, 14.3% did. Days off due to symptoms showed an increasing linear trend with the presence of more respiratory symptoms ($P < .001$). For those who lost one to 3 days, only 15.6% presented with ILI, but for those who took 4 or more days off, up to 41.0% did. There was a significant difference in the proportion of seropositive subjects who took days off (20%) and sought medical assistance (41%), compared to the seronegative group (16% and 34% respectively). Subjects were mostly affected in October (26.0%) and November (24.8%). Approximately 38% of the seropositive subjects were vaccinated against seasonal influenza 2008 or 2009, while 45% of the seronegative subjects were vaccinated ($P < .001$). Vaccination, however, showed an increasing linear trend with age ($P < .001$), but after adjusting for age and sex, the difference was not significant ($P = .83$). None of the subjects reported having received vaccination against influenza A/H1N1/2009 (see Table 2).

Discussion

Our results show that, overall, seroprevalence in our population was 39%. In our previous findings it was significantly higher in subjects younger than 20 years old (49.5%), decreasing as age increased (27.9%) in those aged 41–60 years, and peaking again in those older than 60 years (38.2%).[22] Other authors have demonstrated similar results.[10,23–25] The findings are consistent with serologic analyses of the 2009 H1N1 virus, suggesting that there are some preexisting pandemic H1N1 immune re-

sponses in the elderly; these are present to a lesser extent in younger adults, but are rarely present in children.[26,27] We estimate that the seroprevalence we found is high, since some authors have found different overall community seroprevalence against pandemic H1N1: 13% in Singapore,[28] 21% in Pittsburgh,[24] 26.7% in New Zealand,[29] and 13.8% in Beijing.[30] Others studied the seroprevalence status of particular groups, and found a seroprevalence of 20% in hospital staff in Taiwan[31] and from 25–40% in children and adolescents only in Western Australia.[32]

This higher prevalence in México might partly be explained by the timing of the epidemic. The first reported cases of confirmed influenza A/H1N1/2009 occurred in México 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. Approximately one third of the seropositive subjects were silently infected, while a high proportion of the symptomatic group reported a respiratory illness, and only a small percentage reported an ILI, which implies a mild clinical presentation of the virus. Seropositive patients with only non-respiratory symptoms, might have had a gastrointestinal disease or any other viral disease, and perhaps could be added to the asymptomatic group. The high incidence of rhinorrhea/nasal congestion in this cohort could be explained by infection with pathogens that cause common cold, such as rhinovirus or coronavirus. Fever and cough incidence for the seropositive ILI group was 100%, as these were the criteria to belong to this group. Our seropositive symptomatic subjects were not confirmed as H1N1 patients during their symptomatic period, so the frequency of reported symptoms is different from that presented by confirmed H1N1 populations in other studies in different countries, where the most prevalent symptoms during the acute illness were fever or cough, followed by rhinorrhea, headache, and myalgias.[8,12,27,33–38] Frequency of symptoms such as diarrhea, vomiting, or abdominal pain was also much lower in our population. However, these and other previous studies of the clinical features of pandemic H1N1 2009 have been performed in patients with acute respiratory illness, seasonal influenza, or confirmed H1N1 2009 infection.[5,12,38–42]

Duration of symptoms (4 d), and days off due to illness (2–3 d) were similar between the seropositive and seronegative groups, although people with more respiratory symptoms, in particular those with ILI, took longer to recover. The proportion of seropositive subjects who took days off, and who sought medical assistance was significantly higher than those who were seronegative, which might be due to the fact that more ILI patients were seropositive, and their illness lasted longer and was more severe. Logistic regression showed that no single symptom was a predictor of seropositiveness. Published data to date have shown varying positive predictive values with the use of fever and cough as clinical predictors of H1N1 2009, ranging from 35% to 83%.[12,13,43–47] However, most of these studies have been conducted in the setting of community outbreaks of seasonal or influenza A/H1N1/2009 influenza. The non-significant difference between the seropositive and seronegative groups regarding symptom profile might account for this lack of symptom prediction of seropositiveness. Subjects affected in April and October showed the highest seroprevalence rates, in accordance with the beginning of the first and second waves, respectively. Approximately 38% of the seropositive subjects were vaccinated against seasonal influenza in either 2008 or 2009, while 45% of the seronegative subjects were. Serologic studies have demonstrated that vaccination with recent (2005–2009) seasonal influenza vaccines is unlikely to provide protection against novel H1N1 virus.[26]

The limitation of the study was that the serum samples were drawn in December, and symptoms were reported from April to December 2009. It is unknown when volunteers seroconverted (between April and December 2009) and if any symptoms reported might be linked with the time seroconversion occurred. This fact might have resulted in the absence of a different clinical spectrum between the seropositive and seronegative subjects. Furthermore, the ability to remember non-specific symptoms months after an illness has abated is difficult for most. However, due to the state of awareness and anxiousness of the population regarding infection with influenza A/H1N1/2009 through the year 2009, people most likely would have indeed remembered any symptoms and reported them during the interviews; the more severe and specific the symptoms, the better people would have recalled them. This reality and the large sample size only further strengthen our conclusion that H1N1 is not associated with any pathognomonic symptom that is highly predictive of infection.

Studies show that attack rates based on clinical criteria for acute respiratory illness were higher than rates based on the criteria for ILI, but the incidence of such respiratory illness was poorly correlated with infection confirmed on real-time polymerase-chain-reaction assay or serologic analysis.[39] Estimates derived early in the current pandemic suggested that only 10% of people in developed countries received laboratory confirmation of pandemic H1N1 influenza.[48] More recent estimates are as low as 1.25%.[9,49] Using household studies and modeling, it has been estimated that 30–40% of influenza transmissions occur in households, about 20% in schools, and the remainder in other settings such as workplaces and the general community.[50,51] This might be in accordance with the high proportion of asymptomatic infections among the seropositive subjects in our study group, giving an indication of a rel-

atively "silent" spread of the disease in our population. While asymptomatic individuals are less infective, their role in the spread of 2009 H1N1 cannot be discounted.[29] Our findings underscore the need to continue vigilance both at the community and individual levels to reduce the spread of disease.

Conclusions

Based on the seroprevalence to influenza A/H1N1 in our population, spread of the 2009 H1N1 influenza virus is high in our population. The majority of those infected had a mild illness or no illness at all, and the clinical presentation was quite varied, as demonstrated by our study. No single symptom was associated as predictor of seropositiveness. Influenza A/H1N1/2009 has spread silently in northern México, as the pandemic virus has caused a considerable proportion of both symptomatic and asymptomatic infections.

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