Epidemiologic study of human influenza A(H1N1)pdm09 virus in Yucatan, Southern Mexico

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RESUMEN
Estudio epidemiológico del virus de la influenza humana A(H1N1)pdm09 en Yucatán, Sureste de México

Objetivo. Describir el patrón estacional y la distribución a nivel regional del virus A(H1N1)pdm09 en una región subtropical de México.

Materiales y Métodos. Los datos clínicos y epidemiológicos se analizaron de la base de datos de la Secretaría de Salud. La detección del virus de influenza A(H1N1)pdm09 se realizaron de acuerdo al protocolo del Centro de Control y Prevención de enfermedades de los Estados Unidos (CDC, por sus siglas en inglés).

Resultados. El virus A(H1N1)pdm09 se detectó en el 53% de los casos sospechosos. El grupo de individuos entre 5-29 años fue el más afectado, con 76% de positividad. A nivel regional, la mayoría de los casos (83%) se presentaron en dos localidades, Mérida la capital de estado, y Valladolid. En Yucatán, el virus A(H1N1)pdm09 fue predominante, sin embargo, el virus de influenza AH3 permaneció en circulación.

Conclusión. Se describe el análisis epidemiológico de la influenza A(H1N1)pdm09 en la región subtropical de Yucatán. Este estudio muestra que el virus A(H1N1)pdm09 presentó un patrón diferente de lo observado en el resto del territorio mexicano. No se observaron diferencias en las manifestaciones clínicas con respecto a otras partes del mundo. Sin embargo, los casos fatales fueron más comunes en el grupo de edad de 25-29 años, diferente de la tasa de fatalidad reportada a nivel nacional.

Palabras clave: H1N1, influenza, Sureste de México, Yucatán.

ABSTRACT
Objective. To describe the seasonal pattern and regional distribution of influenza A(H1N1)pdm09 virus within this subtropical region of Mexico.

Materials and Methods. Clinical and epidemiological data were analyzed from a database provided by the Ministry of Health. Influenza virus A(H1N1)pdm09 detections were performed according to CDC protocols.

Results. The A(H1N1)pdm09 virus was detected in 53% of suspected cases. Young individuals between 5-29 years old were the most affected, with 76 % positives. At the regional level, most of the cases (83%) were confined to two municipalities, Merida the state capital, and Valladolid. In the Yucatan outbreak, the A(H1N1) pdm09 virus was the predominant strain, however, influenza A H3 remained in circulation.

Conclusion. We described the epidemiological analyses of Influenza A(H1N1)pdm09 in the...
subtropical region of Yucatan. Our study shows that the influenza A(H1N1)pdm09 virus behaved in a distinct pattern from that observed in the rest of the Mexican territory. No differences in clinical manifestations were observed according to reports from all other locations. We found that fatal outcomes were more common in the age range of 25-29 year olds, differing from the national case-fatality ratio.

**Key words:** H1N1, Influenza, Southern Mexico, Yucatan

**INTRODUCTION**

The recent influenza pandemic caused by the A(H1N1)pdm09 virus was first reported in Mexico City on March 2009. After a strong government-driven mass media campaign promoting hygiene and self/community-protection measures, the epidemic was affected positively and was eventually controlled.

The A(H1N1)pdm09 virus emerged in Southern Mexico by mid-April, then in June 2009, the number of suspected and confirmed cases increased exponentially in Yucatan and Chiapas states. In Yucatan, influenza A(H1N1)pdm09 virus transmission resulted in a large outbreak, which represented an important number of cases, known as “the second wave” of the epidemic in Mexico.

This study presents a comprehensive description of the epidemiology of the Influenza A(H1N1)pdm09 virus in Yucatan, based on unpublished data provided by the local Ministry of Health and by virological analyses performed at the virology laboratory of the Autonomous University of Yucatan (UADY), from April to November 2009. This report’s information includes the detection and characterization of both A(H1N1)pdm09 and seasonal influenza viruses during that period. It also describes the seasonal pattern and regional distribution of influenza A(H1N1)pdm09 viruses within this subtropical region of Mexico.

**MATERIALS AND METHODS**

**Samples from suspected A(H1N1)pdm09 cases collected between April to November 2009.** Clinical specimens were collected from individuals with acute respiratory illness at Primary Health Services (public and private) in Yucatan. Suspected A(H1N1)pdm09 cases in Mexico were defined as: any individual with fever, cough, and headache, including at least one respiratory symptom (rhinorrhea, myalgia, coryza, sore throat, chest pain, abdominal pain, or nasal congestion), and arthralgia. In children under 5 years old, headache was replaced by irritability. Upper respiratory tract samples were taken using throat swabs. In some severe and fatal cases, lung biopsies or bronchial lavage were received on viral transport media.

All samples were tested for influenza A(H1N1)pdm09 at Laboratorio de Virologia, Centro de Investigaciones Regionales "Dr. HideyoNoguchi". Universidad Autonoma de Yucatan.

RNA extraction was performed using either manual (Viral RNA Kit Qiagen) or automated (MagnaPure LC Robot from Roche) methods. Viral RNA was stored at -20°C. Confirmation for influenza A(H1N1)pdm09 was performed according to the protocols and guidelines of the Centers of Disease Control and Prevention (CDC). The protocols also included a panel of oligonucleotide primers for universal detection of type A influenza viruses. This allowed us to identify samples positive to influenza A, but negative to A(H1N1)pdm09 virus.

**Immunofluorescence.** MDCK cells were infected with 100 µl of influenza A positive samples. After incubation and observation of >30% of CPE, cells were harvested and washed 2 times with PBS. Each cell pellet was suspended in PBS, and 20 µl of cell suspension was dropped onto 4 circles of each slide. Cells were fixed with cold acetone for 20 minutes, washed 2 times with...
PBS/Tween 20 and incubated with WHO/CDC monoclonal antibodies against AH1, AH3, and influenza type A or normal mouse antibody as a negative control. After 30 minutes of incubation, slides were washed 2 times with PBS/Tween 20 and incubated with an anti-mouse IgG FITC for 30 minutes. After washing, slides were mounted with a cover slip using mounting fluid. Slides were examined with a fluorescent microscope at 20X for cells exhibiting the green fluorescence.

**Data Analysis.** The analysis of clinical and demographic data presented in this study was performed using a name coded database provided by the local Ministry of Health. Microsoft Excel was used for data input and figures. The statistical descriptive analysis was performed with GraphPad Ver4, and ArcView3.2 for mapping.

**Ethical Considerations.** The laboratory received and analyzed anonymous samples previously coded by the local authorities and the patients’ identities remained confidential. The laboratory was only informed of the clinical status of the patients (mild, severe or fatal cases) to expedite the diagnoses.

**RESULTS**

*The influenza A(H1N1)pdm09 outbreak in Yucatan.* A total of 6142 samples were analyzed between the 1st of April and November 28 of 2009. Fifty three per cent (3231/6142) were positive to influenza A(H1N1)pdm09 virus, using real time RT-PCR. The ratio of total samples available by gender (3084 males and 3058 females) and those positive to A(H1N1)pdm09 virus were close to 1:1 (1632 males and 1598 females).

From all suspected cases analyzed, 93% corresponded to symptomatic outpatients and 7% from inpatients. Influenza A(H1N1)pdm09 virus was confirmed in 52 % of outpatients (2990/5702) and 54 % of inpatients (241/440), referred to a hospital due to diverse clinical complications other than pneumonia. Mortality associated with A(H1N1)pdm09 virus infection was confirmed in 29 individuals, corresponding to 4% of the national A(H1N1)pdm09 - associated mortality during that period.

The age group distribution of positive cases to A(H1N1)pdm09 shows that the most frequently affected were young individuals.

### Table 1

<table>
<thead>
<tr>
<th>Age</th>
<th>Outpatients Positve(%)</th>
<th>Hospitalized Positive(%)</th>
<th>Outpatients Positive(%)</th>
<th>Hospitalized Positive(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4</td>
<td>217 (7.0)</td>
<td>23 (9.5)</td>
<td>22 (10.0)</td>
<td>2 (8.0)</td>
</tr>
<tr>
<td>5-9</td>
<td>496 (16.5)</td>
<td>35 (14.5)</td>
<td>26 (12.0)</td>
<td>2 (8.0)</td>
</tr>
<tr>
<td>10-19</td>
<td>1187* (40.0)</td>
<td>77 (32.0)</td>
<td>67 (31.0)</td>
<td>5 (20.0)</td>
</tr>
<tr>
<td>20-29</td>
<td>603 (20.0)</td>
<td>47 (19.5)</td>
<td>42 (18.0)</td>
<td>7 (28.0)</td>
</tr>
<tr>
<td>30-39</td>
<td>248 (8.0)</td>
<td>17 (7.0)</td>
<td>26 (12.0)</td>
<td>1 (4.0)</td>
</tr>
<tr>
<td>40-49</td>
<td>155 (5.0)</td>
<td>17 (7.0)</td>
<td>15 (7.0)</td>
<td>4 (16.0)</td>
</tr>
<tr>
<td>50-59</td>
<td>645* (2.0)</td>
<td>16. (6.5)</td>
<td>8 (4.0)</td>
<td>3 (12.0)</td>
</tr>
<tr>
<td>&gt;60</td>
<td>19* (0.6)</td>
<td>9 (4.0)</td>
<td>7 (3.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>2990</td>
<td>241</td>
<td>213</td>
<td>25</td>
</tr>
</tbody>
</table>

p <0.05 when outpatients where compared to hospitalized by age group

### Table 2

<table>
<thead>
<tr>
<th>Clinical symptoms</th>
<th>A(H1N1)pdm09 Positive(%)</th>
<th>Influenza A Positive(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>3210 (99%)</td>
<td>233 (98%)</td>
</tr>
<tr>
<td>Cough</td>
<td>3099 (96%)</td>
<td>225 (94%)</td>
</tr>
<tr>
<td>Headache</td>
<td>3034 (94%)</td>
<td>220 (92%)</td>
</tr>
<tr>
<td>General malaise</td>
<td>2738 (85%)</td>
<td>198 (83%)</td>
</tr>
<tr>
<td>Rhinitis</td>
<td>2397 (74%)</td>
<td>177 (74%)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>2157 (67%)</td>
<td>183 (77%)</td>
</tr>
<tr>
<td>Sore throat</td>
<td>1885 (58%)</td>
<td>162 (68%)</td>
</tr>
<tr>
<td>Nasal congestion</td>
<td>1861 (57%)</td>
<td>134 (56%)</td>
</tr>
<tr>
<td>Shivering</td>
<td>1491 (46%)</td>
<td>144 (60%)</td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>350 (11%)</td>
<td>112 (47%)</td>
</tr>
<tr>
<td>Cyanosis</td>
<td>40 (1%)</td>
<td>30 (13%)</td>
</tr>
</tbody>
</table>
Forty per cent of positive subjects were between 10 and 19 years old, 20% were between 20 and 29 years of age, and 16% were between 5 and 9 years old. These age groups were 76% of the total A(H1N1)pdm09 cases in Yucatan. A similar age group distribution was observed in inpatients (Table 1). Higher mortality rates associated to the A(H1N1)pdm09 virus were observed in the 20-29 age group.

Clinical symptoms observed in A(H1N1) pdm09 patients (Table 2), suggests a concordance between the case definition employed by the Mexican Ministry of Health, and the actual symptoms found. Most patients reported fever, cough and headache (94 to 99%). Symptoms like prostration, odynophagia, abdominal pain and conjunctivitis were reported in 20-35% of the positive patients. Dyspnea and cyanosis were the less common (< 15 %).

An interesting clinical observation during the pandemic in Yucatan was a higher incidence of diarrhea; although A(H1N1)pdm09 was not tested in stool samples. 4.5% of positive to A(H1N1)pdm09 reported diarrhea (145/3231), afrom these with 87% of patients during the epidemic peak (120 cases in June, 11 in May, and 8 in July). The remaining cases were one in April, three in October, and two in November. A X2 test was significant for diarrhea and A(H1N1)pdm09 (p = 0.0061).

**Temporal and Geographical distribution.** The first confirmed cases of influenza A(H1N1)pdm09 by the local Ministry of Health of Yucatan were in mid-April 2009 (epidemiological week [EW] 16). Five weeks after the first A(H1N1)pdm09 case was reported in central Mexico, Yucatan only reported 0.4%-2.8% of all confirmed cases in the country during the first wave of the epidemic in Mexico (EW 16 to 19) (Figure 1). However, a rapid increase on the number of cases was recorded by the local Ministry of Health by EW 22 and continued until EW 27 (the second wave of infection). Finally, a third wave occurred in Mexico from late August to mid-November (EW 34-45) (Figure 1). During this period, the number of cases in Yucatan remained relatively low, compared to the cases reported nationally.
The state of Yucatan is located in southeast Mexico, on the Yucatan Peninsula. The peninsula includes the states of Campeche, Quintana Roo and Yucatan (Figure 2). The city of Merida is the capital of the State of Yucatan and is located 1510 Km southeast from Mexico City, the epicenter of the influenza A(H1N1)pdm09 virus outbreak. Yucatan is divided in 106 municipalities, including the metropolitan area of Merida.

The positive cases in Yucatan were distributed across 76% of the municipalities (81 of 106) (Figure 2). However, the municipalities of Merida and Valladolid reported 83% of the total cases recorded between April and November of 2009 (4732 and 289 cases reported in Merida and Valladolid respectively). 2535 (53%) and 158 (54.6%) were confirmed (Figure 2).

Presence of other influenza viruses during the pandemic period. Non-typified Influenza A was detected in 238 individuals, including 25 total hospitalized between the 10-19 and 20-29 age groups (Tables 1 and 2). Influenza typing was assessed in 154 specimens (65%). Influenza AH3 was predominant in 149 samples (79 detected by multiplex RT-PCR and 70 by immunofluorescence {IFI} from infected MDCK cell cultures). Influenza AH1 was identified in only 5 viral isolates.

The influenza AH3/AH1 virus showed a similar temporal distribution as the A(H1N1)pdm09 virus, with the higher peaks of transmission occurring during the second wave (EW 22 to 27) (Figure 3).

**DISCUSSION**

Mexico and the world experienced the first
pandemic of the 21st century in 2009. Mexico City was the epicenter of the outbreak. During the spring of 2009, the A(H1N1)pdm09 virus caused a second and a third wave of infection. The presence of the influenza A(H1N1)pdm09 virus strengthened the surveillance capabilities at the national and state level. It resulted in large-coverage and systematic epidemiological data collection carried out for both pandemic and seasonal influenza viruses. It has been updated weekly by the National and State Ministries of Health9.

In Mexico, the definition of a suspected case was modified according to the development of the epidemic. First, a suspected case was any individual with fever, cough and respiratory distress. Later, the definition was modified: respiratory distress was replaced by headache and included at least one respiratory symptom (rhinorrhea, myalgia, coryza, sore throat, chest pain, abdominal pain, or nasal congestion), and arthralgia. In children under 5 years, headache was replaced by irritability. Overall, our data shows that clinical manifestations of fever, cough and general malaise were a clear identifiers of positivity to the A(H1N1)pdm09 virus, in agreement with other reports10-12.

Positivity to the pandemic virus in Yucatan was more frequent among the age group of 10 to 29 years, for both in and outpatients. Severe and fatal cases occurred in age groups where such clinical outcomes are generally uncommon (mean age of 26 years). The clinical behavior observed in Mexicans affected by the A(H1N1)pdm09 virus showed an increased predisposition in the 15-50 age group. The fatality-case ages ranged from 5 to 45 years13. This result contrasts with data published by Chowell (2011) who reported that Mexican patients older than 60 years had the highest mortality rates14.

This study describes the epidemiology of influenza A(H1N1)pdm09 virus in one of the Mexican states most affected by the second wave of infection. Our study provides additional information about the epidemiology of the disease at the regional level. The most important and populated municipalities reported the highest number of positive cases. Merida, the state capital, represents 42.5% of the total population in Yucatan and contributed with 78% of the cases. Interestingly, Merida connects with eight other state municipalities and has an active daily population movement. This immediacy was not a strong determinant for the number of positive cases. Neighboring municipalities like Kanasin and Uman (with 2.3 and 2.9% of the state population), only reported 0.6 and 1% of the positive cases.

Valladolid (3.8% of total population in Yucatan) reported 4.8% of the total cases. Other important municipalities were Tizimin (4% of the state population) and Progreso (2% of the state population).
population), which reported 2.9% of positive cases. Valladolid is the second most important city of the state and is an important touristic location between Chichén Itzá and Cancun, both important international touristic destinations. Progreso and Tizimin are also important touristic places.

The Mexican epidemic originated at the states of Mexico City, Veracruz, Tlaxcala, and San Luis Potosí. During the first wave of infection the A(H1N1)pdm09 virus extended throughout the Mexican territory. Thirty seven percent of the Mexican states reported 1-50 cases, 28% (including Yucatan) reported 50-100 cases, while 31% reported more than 100 cases. Only Mexico City had more than 1000 cases. During this period, the epidemic concentrated in central Mexico. Later, during the second wave, the epidemic moved to the South, where Chiapas and Yucatan reported more than 2000 cases each. They also reported the highest morbidities, followed by Mexico City. Finally, during the third wave, the epidemic moved to the northern part of Mexico, affecting states during the cold season.

Interestingly, the pattern of influenza circulation in Yucatan was different to the rest of Mexico. The number of cases was remarkably reduced during the third wave, even with the beginning of school activities in late August. We do not have evidence to explain the causes for increased cases in Yucatan during the second wave of the epidemic, however climate conditions such as humidity, temperature, and precipitation need to be further analyzed. From previous reports by our group, the A(H1N1)pdm09 virus followed a similar temporal trend as those observed in previous years for the seasonal influenza in Yucatan.

CONCLUSION

The influenza epidemic caused by the A(H1N1)pdm09 virus in Yucatan represented the second wave in Mexico, and occurred in spite of strict mitigation strategies adopted by the Mexican government. The influenza A(H1N1)pdm09 virus in the subtropical region of Yucatan showed distinctly different patterns from those observed in the rest of Mexico. It only presented one wave of infection. Positive effects from this epidemic include the reinforcement of the prevention, diagnosis, management, and surveillance capabilities of the national and regional health authorities in Mexico.

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