

Profile of the first cases hospitalized due to influenza A (H1N1) 2009 in Panama City, Panama, May-June 2009

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Abstract

Introduction: In April 2009, a novel influenza A (H1N1) virus was identified in patients from Mexico and the United States. From 8 May through 25 June 2009, in the Republic of Panama, 467 cases infected with the same virus were identified, 13 of which were hospitalized at the Santo Tomas Hospital in Panama City. Up to the date of this report, no deaths have been reported in Panama. This study presents the first thirteen cases of Influenza A (H1N1) 2009 that were hospitalized in Panama City.

Methodology: The Santo Tomas Hospital (HST), a third-level institution of the Ministry of Health (MINSa) for adult health care (patients above the age of 14), was designated as the reference center for treating these cases. For this purpose, the norms and criteria established by the system were followed and every patient (case) presenting flu-like symptoms was included (fever equal or greater than 38°C (100.4°F), cough, sore throat, rhinorrhea, lethargy in children under the age of one, and respiratory distress).

Results: Seventy-six patients were hospitalized as suspected cases for infection with the influenza A H1N1 2009 virus, of which 13 (17.1%) were confirmed as positive. The clinical picture was characterized by fever (100%), cough (92.3%), rhinorrhea (69.2%), malaise (53.8%), headache (53.8%), and only one case presented gastrointestinal symptoms (diarrhoea). The male:female ratio was 1:2.2.

Conclusion: The knowledge and technology translation previously acquired through courses to the HST health care providers were the key in controlling the first influenza A (H1N1) 2009 cases.

Key words: influenza, fever, virus, pandemic, Panama

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Introduction

From 15 through 17 April 2009, the Center for Disease Control of the United States (CDC) identified two cases of human infection by a novel type of influenza A (H1N1). This novel virus presented genetic characteristics that had not been previously identified, in influenza A in humans, swine or poultry [1,2]. By mid-April, Mexico confirmed a considerable number of cases of respiratory disease caused by this virus, thus triggering an alarm that resulted in economic and political effects on that country [3]. On 24 April, the World Health Organization (WHO) issued an official statement declaring a public health emergency of international interest, and on 11 June, the pandemic alert level increased to phase 6, indicating that the human-to-human transmission of the virus had occurred in at least two countries of two different WHO regions. The Ministry of Health of Panama (MINSa per its Spanish acronym) registered the first case of influenza A (H1N1) 2009 on 8 May 2009. By

25 June 2009, the number of confirmed cases reached 467 (176 adults, 291 children) of which 13 (7.4%) adults were hospitalized [4,5]. To date, no deaths have been reported in Panama. In this study, we present the first thirteen cases of influenza A (H1N1) 2009 that were hospitalized in Panama City.

Methods and materials

Due to the alert established by the WHO after cases were reported in several countries, MINSa implemented management strategies (triages, hospitalization, outpatient management, diagnosis, treatment, and logistics) and a pro-active search for contacts of suspected, probable and confirmed cases. The Santo Tomas Hospital (HST per its Spanish acronym), a third-level institution of the MINSa for adult health care (patients above the age of 14), was designated as the reference center for treating these cases. For this purpose, the norms and criteria established by the system were followed and patients (cases) presenting flu-like symptoms such as fever

equal or greater than 38°C (100.4°F), cough, sore throat, rhinorrhea, lethargy in children under the age of one, and respiratory distress were included.

A suspected case was considered when a patient, besides having the above-mentioned symptoms, presented any of the following during the previous 10 days: history of travelling to any of the countries with confirmed cases or contact with persons arriving from these countries; or contact with a confirmed case in the national territory; or having been in some area of the country with confirmed cases. Initially, all symptomatic suspected cases were hospitalized, whether arriving at the Emergency Room of the HST or referred from another hospital facility (public or private). In addition, all symptomatic contacts of a confirmed case were hospitalized. Two nasopharyngeal swabs were taken from all suspect cases, one for doing the rapid test for Influenza (immunochromatography) at the HST laboratory and the other one to be sent to the Gorgas Memorial Institute of Health Studies (ICGES per its Spanish acronym), the National Influenza Center for confirming the diagnose. All suspected cases were immediately reported to the MINSA.

The diagnosis of influenza A H1N1 was conducted following the protocol recommended by the CDC. Consequently, the RNA was extracted using a commercial kit (RNAeasy, QIAGEN), real-time PCR (RT-PCR) with primers and probes for influenza A, swine flu A, swine H1 and RNaseP (SuperScript III Invitrogen) in an ABI 7300 thermal cycler. Over 50% of the positive samples were confirmed through viral isolation at ICGES and sent to the CDC. RT-PCR test results on original specimens and grown viral cultures (confirmed at the CDC) indicated all these specimens to be positive for influenza A swine and negative for human seasonal influenza A/H1, A/H3. The neuraminidase inhibition assay was used to assess the susceptibility of the isolates to neuraminidase inhibitors, finding them to be resistant to the adamantanes. In addition, the sequence analysis was performed for resistant viruses to reveal the presence or absence of an established molecular marker of resistance to the neuraminidase inhibitor (S31N in M2).

Nasal swab samples from patients with confirmed H1N1 diagnoses by RT-PCR were used for viral isolation in MDCK cells lines following standard procedures. Viral supernatant obtained from cell cultures were used for RNA extraction using Qiamp RNA Viral Minikit following the manufacturer's instructions. Reverse transcriptase

PCR reaction was performed using AccessQuick RT-PCR kit (Promega) to amplify Neuroaminidase (NA) and Hemagglutinin (HA) genes with primers SFHA-1 GGA ACG TGT TAC CCA GGA GA and SFHA-2 CCT GTG GCC AGT CTC AAT TT, and primers SFNA-1 TGG CAT CAA TTG GCT AAC AA and CTC CCG CTA TAT CCT GAC CA, respectively. PCR conditions were the same for both genes, as follows: first cycle of 42°C for 30 minutes, one cycle of 95°C for 3 minutes, 35 cycles for 95°C for 20 seconds, 53.5°C for 30 seconds, 72°C for one minute and, one cycle of 72°C at 10 minutes. Amplicons were prepared for sequencing by agarase digestion on agarose isolated DNA-PCR bands of expected size. Direct cycle sequencing reactions were performed to each amplicon using standard procedures with BigDye Terminator (v3.1). Sequencing reaction products were resolved on a sequencer ABI3130 (Applied Biosystems). Sequence data was assembled and contiguous sequences were generated with Sequencher Software v.4.7 (GeneCodes). Sequences generated were aligned to Genbank sequences (accession numbers CY047715- CY047740) for phylogenetic analysis using ClustalX and Mesquite softwares.

Phylogenetic trees were inferred with the use of Bayesian method based on the General Time Reversible (GTR) + I+ γ 4 substitution model and bootstrap analysis in Mr. Bayes software. Generated trees were visualized in TreeView and Mesquite softwares.

The demographic, clinical information, and risk factors were obtained from the clinical files of confirmed cases of influenza A (H1N1) 2009. Additional information was obtained through interviews with patients, using the corresponding personal protection measures. The remaining epidemiological information regarding the case's contacts was collected by the MINSA's Epidemiology Department. The information was recorded and analyzed using the EpiInfo™ Program Version 3.5.1, 2008, respecting the pertaining ethical norms of confidentiality and privacy.

Results

Between 8 May and 25 June 2009, seventy-six patients were hospitalized as suspected cases for infection with the influenza A (H1N1) 2009 virus, of which 13 (17.1%) were confirmed as positive. All patients came from different counties—i.e., *corregimiento*, the smallest political division—of the province of Panama, Panama, and the mean age was

Table 1. Sociodemographic and clinical characteristics of influenza A H1N1 2009 and seasonal influenza in hospitalized patients. Santo Tomas Hospital. Mav-June 2009.

Characteristics	Influenza	
	H1N1 2009 No. (%)	Seasonal** No. (%)
<i>Gender</i>		
M	4 (30.8)	2(22.2)
F	9 (69.2)	7(77.8)
<i>Age</i>		
15-24	3 (23.1)	2(22.2)
25-44	8 (61.5)	2(22.2)
45-64	2 (15.4)	5(55.6)
65 and more	0	0
<i>Risk factors</i>		
Travel to USA	3 (23.1)	
Travel to Colombia	1 (7.7)	
Contact with index case*	4 (30.8)	
Contact with positive cases	2 (15.4)	
Without information	3 (23.1)	
Median of the beginning of symptoms (days)	2.6	2.1
Range of the beginning of symptoms (days)	1 to 6	1 to 8
<i>Symptoms</i>		
Fever	13 (100)	9(100)
Cough	12 (92.3)	9(100)
Rhinorrea	9 (69.2)	6(66.7)
Malaise	7 (53.8)	6(66.7)
Headaches	7 (53.8)	5(55.6)
Myalgia	3 (23.1)	3(33.3)
Sore throat	3(23.1)	6(66.7)
Respiratory distress	1 (7.7)	0
Diarrhea	1 (7.7)	1(11,1)
Chills	1 (7.7)	0

*Index Case: the first case detected in the country.

**Fisher's exact test: all the values were not statistically significant ($P > 0.05$).

26 years (range: from 20 to 54 years). The clinical picture was characterized by fever (100%), cough (92.3%), rhinorrhea (69.2%), malaise (53.8%), headache (53.8%), and only one case presented gastrointestinal symptoms (diarrhoea). The male:female ratio was 1:2.2. As shown in Table 1, the clinical picture of seasonal influenza was similar with no statistical significance (Fisher's exact test $P > 0.05$). The complete blood count data (hemoglobin, hematocrit, leucocytes, and platelets) of the 13 patient were in the normal range and chest X rays

were unremarkable. The liver enzymes (AST, ALT) were slightly elevated in the patients.

Patients were hospitalized in the Infectology Ward and none of them required admission to the Intensive Care Unit or intubation. The median days of hospitalization was 2.6 days and the range was between 1 to 6 days. Three women were between 24-26 weeks of pregnancy. Two patients had to be readmitted to the hospital due to complications: case 1 presented mild hemoptysis and case 9 had deterioration in her general condition. Both patients

Figure 1. Follow-up of first eleven hospitalized patients. Santo Tomas Hospital, 2009. *Pregnant women

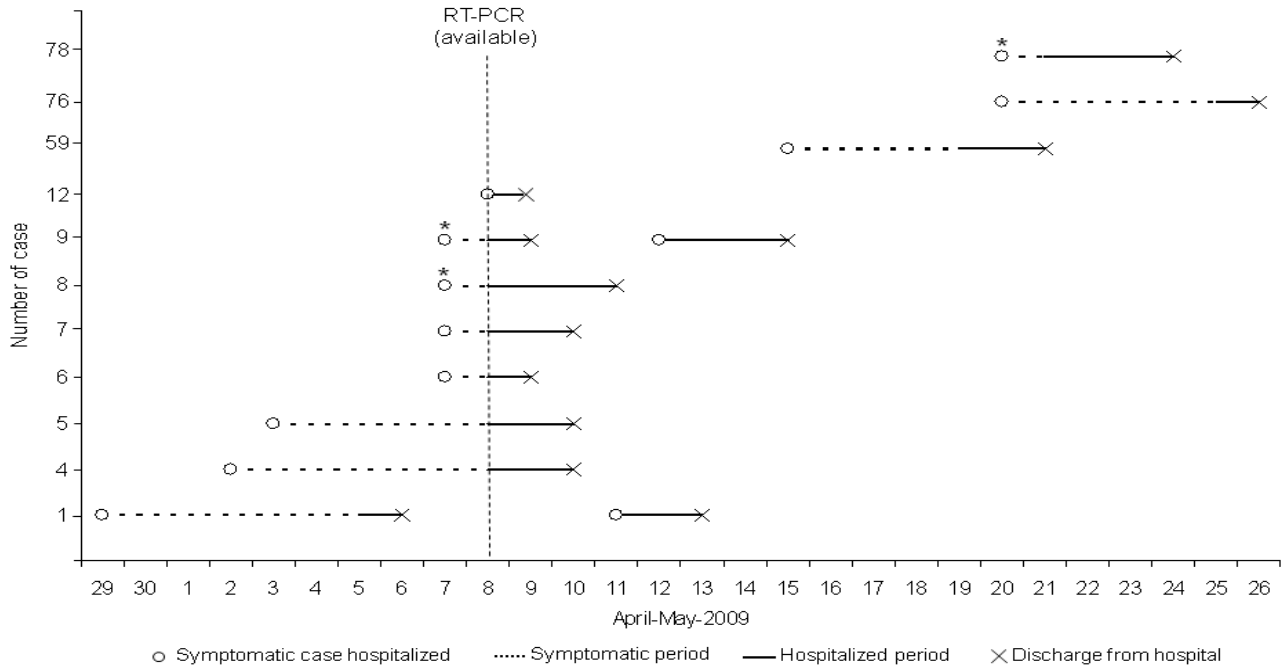
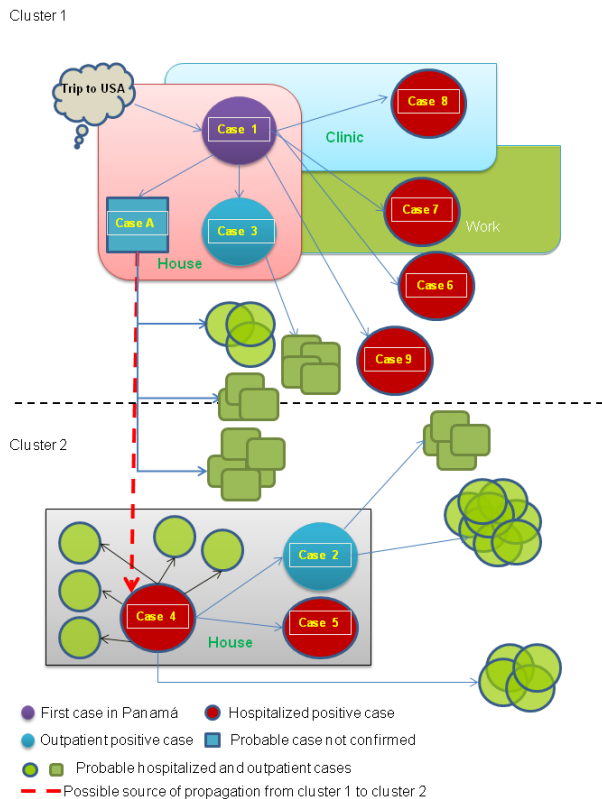


Figure 2. Hypothesis of the evolution of Influenza A (H1N1) 2009 cases.



The first case (index case) was a 25-year-old male patient from Panama City with history of travelling to the United States. Ten days after arriving at Panama, the patient started having fever and rhinorrhea and visited a private clinic where he was referred to the HST. A nasopharyngeal swab was taken which was initially negative for influenza A. Two days later, the RT-PCR test (available since 8 May 8) for influenza A H1N1 gave a positive result. Cases 3, 6, 7, 8 and 9 were direct contacts of the index case.

Case A: Father of Case 1 who presented mild symptoms (after his son returned from the United States) that resolved without complications and therefore did not seek medical attention. Two weeks after symptoms resolved, the test was performed with negative results. This case was not included in our study since he was not hospitalized but was important for understanding the epidemiological progression of the disease.

Case 2: Eight-year-old boy, residing in the suburbs of Panama City, with history of asthma; no relationship to the cases described earlier. He came to the hospital (Children's Hospital) due to fever and lethargy. As part of the routine screening, a sample was taken and the results were positive for the novel virus.

Case 3: Index case's sister, asymptomatic, was not hospitalized. Research was carried out and the case was confirmed since she had had contact with a confirmed case.

Case 4: Case 2's sister who worked in customer service at a telephone company in Panama. She had become sick a few days before with intense fever and malaise. This patient had contact with Case A when he went to pay his telephone bill at the company where she worked.

Case 5: Case 4 and Case 2's mother. She presented fever, cough and odynophagia and tested positive.

Cases 6 and 9: Direct contacts of the Case 1. They presented symptoms, tested positive, and were hospitalized.

Case 7: Personal assistant of Case 1. Case 7 presented fever, rhinorrhea and cough and attended a hospital when a sample was taken and tested positive.

Case 8: Pregnant woman who visited a private clinic on the same day Case 1 did. This is where she had contact with him. After two days, she presented fever, rhinorrhea and headache and attended a hospital where a sample was taken and tested positive.

recovered 48 hours after their re-admission (Figure 1).

Our hypothesis was that there were initially two clusters for the transmission of the disease. In Cluster 1, six confirmed cases were identified, of which five were hospitalized (including the index case). In Cluster 2, three confirmed cases were identified of which two were hospitalized. Case A is the probable connection between the two clusters (Figure 2).

Finally, none of the confirmed cases in Panama (influenza A (H1N1) 2009) presented in this study was fatal and all, as well as their symptomatic contacts, were treated with Oseltamivir.

Preliminary phylogenetic analysis of HA and NA genes with published sequences of influenza A H1N1 from the same epidemiological period (May 2009) suggests that circulating variants in 2009 are more related to isolated strains from California (samples 04, 05, 06, 07 from 2009), Texas (samples 04 and 06 from 2009), Ohio (sample 07 from 2009), and New York (sample 19 from 2009).

Discussion and Conclusion

As of 25 June 2009, a total of 94,512 confirmed cases of influenza A (H1N1) 2009 had been reported in more than 100 countries in the world and Panama had confirmed 467 cases [5]. Furthermore, 17% of the suspected cases considered in this study were found to be positive. Young adults were more frequently affected and the female gender slightly predominated but this had no statistical significance (Fisher's exact test $P > 0.05$). Symptoms in most of the patients were in accordance with those described in the literature and were similar to those observed in seasonal influenza [2,6-8]. In this study, the mean of hospitalization days was high because at the beginning of the outbreak, the progression of the disease was not fully understood and rapid control was needed. Therefore, all the patients with symptoms (no matter the severity) were hospitalized in HST. Sixty-nine percent (324) of the cases detected by the surveillance system came from the province of Panama, which supports our hypothesis that cases disseminated from the clusters described above (*i.e.*, Case A was the possible source of dissemination from Cluster 1 to Cluster 2) and that the H1N1 influenza virus was introduced into the country by a patient who had recently been infected in the United States [1].

The HST, as a national referral center, had been selected since 2007 to handle a possible influenza viral pandemic. Toward this end, strategies endorsed

by the MINSA were established; these included training health care professionals and management as well as developing medical care guidelines. Due to the worldwide alarm announced by the WHO, in order to face this event, Panama activated the system based on experience previously acquired, and thus the HST played a key role in managing the first hospitalized patients.

The disease has been described as a mild respiratory infection and no cases in Panama have had serious complications or have been fatal, coinciding with most cases reported in the literature [2,8]. As a matter of fact, nine (12%) of the total cases of this study were positive for seasonal influenza that is clinically undistinguishable from the confirmed cases of the novel virus ($P > 0.05$). In this sense, we consider that the country should continue with active surveillance measures to face this public health emergency.

We believe that knowledge and technology translation previously acquired through courses to the HST health care providers were the key in controlling the first Influenza A (H1N1) 2009 cases.

Phylogenetics analysis showed that influenza A H1N1 variants circulating in May 2009 were more closely related to variant isolates from the United States (California, Texas, New York, and Ohio) and probably were the origin of different epidemiologic clusters of transmission. As expected, variants circulating in 2009 were not related to published variants from 2008 (A/Panama/1310/2008(H1N1)) which were closest to isolates from Brisbane (A/Brisbane/59/2007(H1N1)) and New Caledonia (A/New Caledonia/20/1999(H1N1)).

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