MONITORING AND CHARACTERIZATION OF EMERGENT INFLUENZA A H1N1 2009 VIRUSES IN RABAT, MOROCCO DURING 2009 - 2010

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Abstract

Background. Immediately after the outbreak of novel influenza A (H1N1) in the United States of America and Mexico, World Health Organization declared a global pandemic (pH1N1). A global monitoring system was set up in Morocco to control the dissemination of the virus and its impact on the public health. This study aims to describe the epidemiological and clinical characteristics of influenza like illness (ILI) patients and confirmed pH1N1 cases diagnosed in the Research and Analysis Medical Laboratory (LRAM) between November 23rd 2009 and March 7th 2010. **Methods.** A total of 300 nasopharyngeal swabs from ILI patients were screened for seasonal influenza A and pH1N1 viruses by real-time RT-PCR. For each patient, clinical and epidemiological information were recorded. **Results.** pH1N1 was confirmed in 149 ILI patients (49.7%). This study showed a peak in week 50/2009. The mean age of pH1N1 cases was 13.4 years and children between 5 and 14 years are the most affected cases (82/149). The most common symptom was the cough reported in 83% of pH1N1 positive cases, followed by headache and asthenia. Among the 149 pH1N1 confirmed cases, 10 had asthma and all of them are children. **Conclusion**. During the pandemic period, pH1N1 was the predominant

influenza A subtype. This study indicates that pH1N1 was widespread and the highest risk group is the children of school-going age (aged 5–14). These results can be used for future planning strategies for influenza and strengthening the influenza monitoring system for better management of the infection and limiting the development of complicated diseases.

Keywords: Influenza A, pandemic A/ H1N1, Symptoms, Monitoring

Introduction

Influenza virus is a common human pathogen that has caused serious respiratory illness and death over the past century. It always had potential to cause widespread pandemics whenever a new type of Influenza strain appeared in the human population and then spread easily from person to person (Khanna et al., 2008).

Since the emergence of a novel influenza A/H1N1 in April 2009 in Mexico and the United States, sustained transmission worldwide occurred rapidly. This new influenza virus arose through the reassortment of a North American triple-reassortant swine influenza virus and a Eurasian swine influenza virus (Smith et al., 2009; Kuchipudi et al., 2009; Peiris et al., 2009; Lalle et al., 2010).

According to the World Health Organization (WHO) clinical definition, an influenza infection is an acute febrile respiratory illness with influenza-like symptoms including at least two of the following symptoms: fever > 38°C, and cough or sore throat in the absence of other diagnoses) (WHO, 2009a). According to the US CDC criteria (Centers for Disease Control and Prevention, Atlanta, USA), influenza infection was defined as fever ≥37.8 °C accompanied by at least one of the following respiratory symptoms: cough, sore throat, headache or muscle ache (CDC, 2009).

The WHO issued alerts about the emergence of pandemic (H1N1) on April 23, 2009. Less than 2 months later, WHO declared pandemic alert stage 6 on June 11, 2009 (WHO, 2009b). Immediately after the alerts, a national mandatory notification system was started in Morocco for influenza-like illness (ILI) cases and hospitalized persons with pH1N1 in public and private institutions. Among The institutions assigned to be in charge of the diagnosis of pH1N1 were National Institute of Hygiene, Pasteur Institute of Morocco, The military hospital Mohammed V and the Research and Analysis Medical Laboratory (LRAM, Fraternelle de la Gendarmerie Royale). In the end of this study (10 March, 2010) 2.890 cases of influenza pH1N1have been identified and 63 have died (Moroccan Ministry of Health, 2011).

The present study was planned to evaluate the prevalence and trend of emergence of pH1N1 virus in ILI cases diagnosed in the LRAM during

the epidemiologic period (November 23rd 2009 to March 7th 2010), and to describe the associating clinical symptoms of patients studied.

Materials and methods

Specimens

In this study, 300 clinical samples of nasopharyngeal swabs were recruited. Samples were collected from patients with influenza-like illness visiting the LRAM from Rabat region in Morocco. Influenza infection was defined according to WHO criteria. For all recruited patients, information on age, sex, date of specimen collection, illness symptoms and date of onset, and administration of antiviral treatment were recorded on appropriate questionnaire. All patients confirmed with pH1N1 were contacted and received a anti-influenza treatment.

RNA extraction from clinical samples

RNA extraction from clinical samples was performed using the QIAamp Viral RNA Extraction mini Kit (Qiagen, Germany), according to the manufacturer's instructions,

RT PCR for screening of influenza pH1N1 viruses

The One step real-time RT-PCR was performed at an 7500 Fast Real-Time PCR System (Applied Biosystems) according to the CDC protocol for the identification of swine influenza A(H1) viruses revision 2 (WHO, 2009c).The reagents and positive control material were kindly provided by CDC.

Each RNA sample was tested for four sets of genes, which are: matrix protein gene segment for the identification of influenza A viruses, nucleoprotein gene segment for the identification of swine A influenza viruses, haemaglutinin gene segment for the subtyping of swine A H1 viruses. Lastly, RNAse P gene segment was amplified to test the RNA extraction procedure, as well as the specimen content of human amplifiable genetic material and inhibition (WHO, 2009c).

Statistical analysis

Statistical analysis was conducted using SPSS statistics 21, Fisher's exact test was performed for statistical analysis, and differences were considered significant for $P < 0.05$. Odds ratio (OR) with Pearson's chi-squared test was used to show the significant age group, keeping 0–4 years as the comparison age group.

Results

During the study period, from week 48/2009 to week 9/2010, swab samples from 300 patients suspected of contracting influenza were tested. Pandemic influenza A pH1N1 was detected in 149 cases (49.7%) whereas seasonal influenza A viruses were detected in only 14 cases (4.6%). Of the 149 pH1N1 positive cases, 81 (54.40%) were women and 68 (45.60%) were men (M/F ratio 0.9:1).

At the beginning of the study on November 23rd 2009, 23 pH1N1 positive cases (15.4%) were registered the week 48/2009. Number of cases increased and reached the peak during the week 50/2009 with 58 pH1N1 positive cases (38.9%) and then decreased progressively (Figure 1).

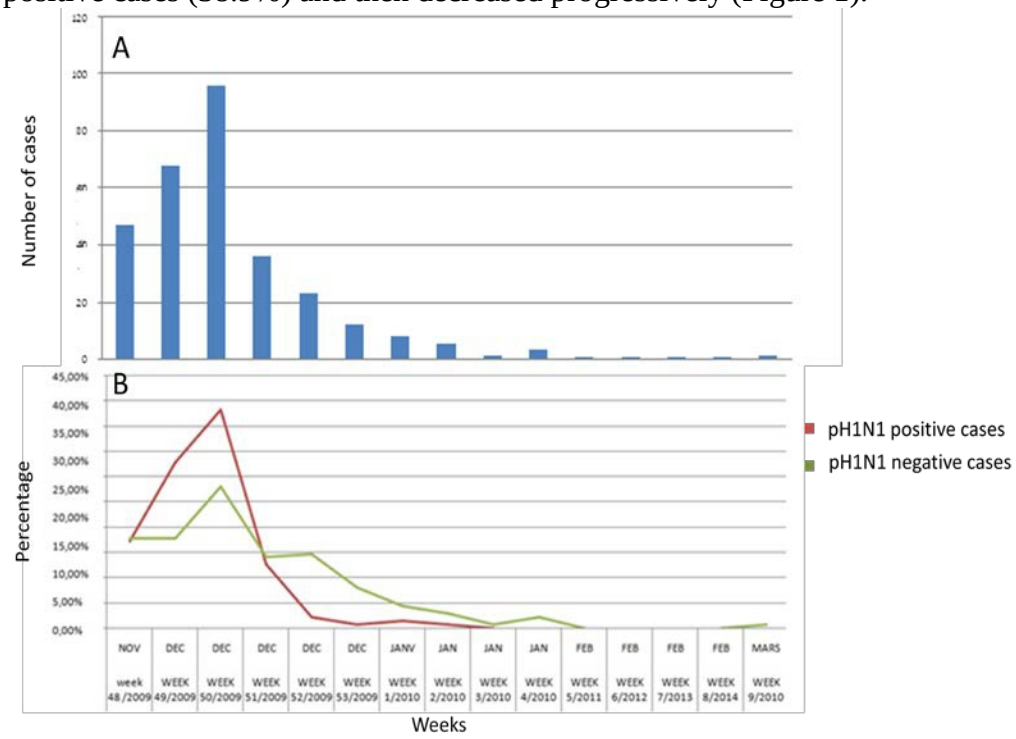

Figure 1: Distribution of pH1N1 confirmed and negative cases during the pandemic period from November 23rd 2009 to March 7th 2010

A: Number of recruited ILI cases.

B: Percentage of pH1N1 positive and negative cases. Percntages were calculated according to total pH1N1 positive and negative cases registered during the pandemic season

Interestingly, during the first weeks of pandemic, the rate of detection of pH1N1 positive cases among ILI patients was higher than the rate of negative cases. After the week 51/2009, the opposite side was observed and ILI patients were diagnosed as pH1N1 negative.

Patients confirmed with pH1N1 ranged from 2 months to 72 years old. The means age of ILI and pH1N1 confirmed patients were 19 and 13.4 years respectively. Distribution of pH1N1 infected cases according to age is reported in Table 1 and clearly showed that children between 5 and 14 years are the most affected by pH1N1 : 82⁄149 (55%, p = 0, OR = 5.25, CI 95% 2.68-10.27). Of particular interest, 25 children less than 4 years were confirmed pH1N1 positive representing 16.78% of total positive cases (Table I).

Table I: Distribution of suspected and pH1N1 cases according to the age

Age Group (years)	Suspected cases		pH1N1 positive cases		P	OR	95% CI	
	N	%	N	%				
< 4	65	21.67	25	16.78	-	-	---	---
5-14	107	35.67	82	55.03	0.000	5.248	2.683	10.266
15- 21	24	8.00	15	10.07	0.047	2.667	1.015	7.004
22- 40	65	21.67	21	14.09	0.464	0.764	0.371	1.571
41- 60	31	10.33	5	3.36	0.032	0.308	0.105	0.906
> 60	8	2.67	1	0.67	0.179	0.229	0.027	1.970
Total	300		149					

Odds ratio; **CI:** confidence interval; *Chi-squared test: **p** = 0.05

Clinical symptoms of all suspected cases and patients with pH1N1 infected cases are reported in table II. The most common symptom was the cough reported in 83% of pH1N1 positive cases. Headache and asthenia were reported respectively in 57.7 % and 52.3 %. Interestingly, only 31 % of pH1N1 infected patients had fever.

Table II. Assessment of clinical symptoms of patients with influenza-like illness and patients infected with influenza pH1N1 virus.

Symptoms	Ili patient (N = 300)		pH1N1 patients (N = 149)	
	N	%	N	%
Fever	62	21%	46	31%
Cough	209	70%	123	83%
Headache	144	48%	85	57%
Asthenia	138	46%	78	52%
Sore throat	91	30%	43	29%
Muscle ache	91	30%	50	34%
Vomiting	41	14%	26	17%
Diarrhea	26	9%	10	7%

Analysis of association between some risk factors and the infection with pH1N1 is reported in Table III. Among the 149 pH1N1 cases, only 15 (10.06%) had at least one risk factor and the most prevalent were asthma reported 10 patients (6.71 %), allergic rhinitis in 3 cases and pregnancy in 2 cases. Of particular interest, all the asthmatic cases are children.

Table III. Risk factors distribution among ILI patients and confirmed pH1N1 cases

	Ili patient N = 300	pH1N1 patients N = 149
Risk Factors	No	No
Asthma	20	10
Pregnancy	5	2
Allergic rhinitis	3	3
Diabetes	3	0
Cardiopaty	3	0
Renal failure	1	0
obesity	1	0

Discussion

During the world pH1N1 pandemic, many epidemiologic studies were carried out worldwide to characterize the epidemiology of the virus to establish the global and specific maps of pH1N1 distribution, to elaborate specific countermeasures to be implemented.

Of particular interest was the early apparition of pH1N1 infected cases in North African countries. The first laboratory-confirmed pH1N1 case in Morocco was identified on June 10, 2009 in a 19-year-old woman travelling from Canada to Morocco, (Barakat et al., 2012). On December 24th 2009 Morocco counted 2.806 cases of influenza pH1N1 and 45 have died. (Moroccan Ministry of Health, 2011). In Tunisia, 1181 admissions to hospital with severe illness have been reported and 170 confirmed cases of influenza pH1N1 with severe infection requiring hospital management in intensive care. A total of 28 pandemic H1N1 influenza-associated deaths were confirmed, and died from a clinically compatible illness or complications attributable to this infection (El Moussi, et al., 2013)

In this field, Morocco has implemented a national surveillance program to monitor the spread of pH1N1 and its impact on the public health. In the course of the surveillance study we conducted during the pandemic period, November 23rd 2009 to March 7th 2010, pH1N1 virus was not the only circulating strain detected. pH1N1 virus was identified in 49.7% of cases and seasonal influenza A viruses were detected in 4.6% of cases. The frequency of pH1N1 in our study population is in agreement with previous reported data in Morocco and worldwide. Indeed, Lahlou and coll. have detected pH1N1 in 40% of ILI patients (Lahlou, 2011). The prevalence of pH1N1 depends on the proximity of the origin sites of the pandemic and the close relationships between them. In Chile, 80% of ILI cases corresponded to pH1N1 (Dabanch et al., 2011). In the Mediterranean region, the frequency of pH1N1 varied between 30 and 50%. Rizzo et al. have reported that 40% of medically attended ILI cases in August 2009 in Italy were pH1N1 positive (Rizzo et al., 2010). Another study conducted in Portugal showed that 30.8%

of analyzed ILI patients were confirmed for pH1N1 (Duque et al., 2010). In France, many studies were conducted upon the pandemic is declared, and the peak of detection of pH1N1 in ILI patients (47%) was obtained in November 2009 (Lainé et al., 2010; Malato et al., 2011).

In some countries, during the pandemic period, pH1N1 was the only identified and isolated virus among all ILI patients. However, after the pandemic period, other influenza viruses, like A(H3N2) and type-B viruses, were detected (Dapat et al., 2012).

Before the pH1N1 pandemic, the viral molecular detection was rarely applied for influenza diagnosis. In our laboratory, influenza detection and pH1N1 identification have started by the mid-November and the first ILI cases recruited were in the week 48/2009. Distribution of recruited ILI patients and pH1N1 confirmed cases presented a peak in the second week of December 2009 and the number of registered cases decreased significantly. Other referent centers in Morocco and the global pandemic surveillance program have reported the same results, and confirm the disappearance of pH1N1 in favor of seasonal influenza (Barakat et al., 2012; Lahlou et al., 2011; Moroccan Ministry of Health, 2011).

Worldwide, there is evidence that age was a risk factor for severe influenza and death. Patients >60 years of age and <5 years of age were at a higher risk for severe disease, and patients >60 years of age were at a higher risk for death. At least 1 concurrent condition was identified as a risk factor for death from pandemic (H1N1) 2009. In this study, the highest rate of pH1N1 positivity was observed among children under 14 years old, persons ≥60 years of age were least affected. These results are in agreement with reported data worldwide. In the USA 60% of the pH1N1 confirmed patients were less than 18 years (Kwan- Gett et al., 2009), whereas in Japan, 88.5% of cases of 2009A /H1N1 were reported in persons <20 years of age, (Kamigaki et al., 2009). In United Kingdom, children under 16 years were more susceptible to infection in the households (Ghani, et al., 2009). In Mexico, as for other Latin American countries, pH1N1 was more predominant in younger people less than 15 Years (Perez-Padilla et al., 2009). The high frequency of pH1N1 among younger people is mainly due to the high exposure rates among school-aged children

Of particular interest, limited number of elderly people was suspected to have influenza and among them only one case was confirmed positive for pH1N1. This hypothesis is supported by subsequent studies (CDC, 2010). Many studies have reported that during the influenza pandemic, risk for illness in this age group was low but risk for severe disease and death was higher than in the other groups, independent of underlying diseases (Dabanch et al., 2011).

In contrast to seasonal influenza, severe disease from pandemic H1N1 seems concentrated in older children and young adults, with almost no cases reported in patients older than 60 yrs, suggesting partial immunity to the virus in the older population (Rothberg et al., 2010).

The most common symptoms of patients with pH1N1 were cough (81.9%), fever (59.7%), headache (57.7%), Asthenia (52.3%), sore throat (34.2%), muscle pain (33.6%), vomiting (17.4%) and diarrhea (6.0%). The same symptoms were reported for pH1N1 patients and ILI patients infected with other viruses, and clinical symptoms of pH1N1 are typical to the seasonal influenza.

Worldwide, Fever and cough are the most reported symptoms. Indeed, Fever was reported in 56 to 100% of pH1N1 infected cases, whereas cough was reported in 54 to 90 % of cases (Biswas et al., 2010; Al Mazroa et al., 2010; Humne, A. Y. et al., 2013; Lessler et al., 2009). Clinical symptoms and their aggressiveness must be taken with precaution. In fact, it's widely accepted that clinical signs can vary significantly according to the age of the patient and the presence of underlying diseases.

Differences in clinical features between influenza A H1N1, A H3N2, and B in adult patients was already reported and showed that fever, headache, general malaise and sore throat were equally frequent in both of them (Kaji et al., 2003). Thus, clinical symptoms are not a good marker for good discrimination between pH1N1 and seasonal influenza.

Moreover, it's widely accepted that some factors may be at risk for contracting influenza viruses and developing complicated diseases such as pneumonia which is the most complicated diseases of the H1N1 outbreak strain. In this field, we have investigated the association between pH1N1 infection and some risk factors. Interestingly, among the 149 pH1N1 positive cases, asthma, allergic rhinitis and pregnancy were the only reported risk factors and were present in 10, 3 and 2 cases, respectively, and all asthmatic cases are the children. These results are in agreement with previous studies that have clearly showed the high significant risk factor for severe disease in children with asthma (O'Riordan et al., 2010). Moreover, patients having underlying conditions, such as respiratory diseases including asthma, require immediate hospitalization (Jain et al., 2009).

Worldwide, pregnancy is also reported as a major risk factor for contracting pH1N1 and developing severe influenza disease. Because of their vulnerability, particular attention must be done to pregnant women, especially in their second and third trimester, and treatment with anti-influenza drugs have to be applied promptly (Jamieson et al., 2009).

Conclusion

During the 2009-2010 influenza season, pH1N1 was the predominant virus subtype in Morocco, and the highest rate of pH1N1 infection was in children under 14 years. The limited numbers of suspected cases with influenza like illness and pH1N1 confirmed cases is mainly due to the efficacy of the established monitoring strategy of influenza surveillance in Morocco and efficient management of infected cases limiting the dissemination of the virus in the whole population. Moreover, intense media coverage that accompanied the pandemic has contributed to enhance the awareness of the whole population on the disease and to inform them on how to protect themselves against the virus, reducing the spread of the epidemic.

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