

# Pandemic 2009 influenza A (H1N1) infection among 2009 Hajj Pilgrims from Southern Iran: a real-time RT-PCR-based study

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**Background** Hajj is a mass gathering undertaken annually in Mecca, Saudi Arabia. The 2009 Hajj coincided with both the pandemic influenza A/H1N1 2009 (A(H1N1)pdm09) and seasonal types of influenza A viruses. The interaction between pandemic influenza and Hajj could cause both a high level of mortality among the pilgrims and the spread of infection in their respective countries upon their return home.

**Objective** The present study attempted to determine the point prevalence of A(H1N1)pdm09 among returning Iranian pilgrims, most of whom had been vaccinated for seasonal influenza but not A(H1N1)pdm09.

**Methods** Pharyngeal swabs were collected from 305 pilgrims arriving at the airport in Shiraz, Iran. RNA was extracted from the samples and A(H1N1)pdm09 and other seasonal influenza A viruses were detected using TaqMan real-time PCR. For A(H1N1)pdm09-positive samples, the sensitivity to oseltamivir was also evaluated.

**Results** Subjects included 132 (43.3%) men and 173 (56.7%) women, ranging in age from 24 to 65 years. The A(H1N1)pdm09 virus was detected in five (1.6%) pilgrims and other influenza A viruses in eight (2.6%). All the A(H1N1)pdm09 were sensitive to oseltamivir.

**Conclusions** Only five cases were found to be positive for A(H1N1)pdm09, and it seems unlikely that the arrival of infected pilgrims to their homelands would cause an outbreak of a new wave of infection there. Thus, the low morbidity and mortality rates among the pilgrims could be attributed to the characteristics of A(H1N1)pdm09, which causes morbidity and mortality in a way similar to the seasonal influenza infections, absence of high-risk individuals among the Iranian pilgrims, and the instructions given to them about contact and hand hygiene, and respiratory etiquette.

**Keywords** Hajj, influenza A/H1N1, mass gatherings, pandemic, polymerase chain reaction, travel.

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## Introduction

Hajj is the largest annual recurring religious mass gathering (MG) in the world occurring in Mecca, Saudi Arabia. More than 2.5 million people from at least 180 countries participate simultaneously in this MG.<sup>1</sup> Such a MG can raise the risks of public health issues such as respiratory infections, meningococcal outbreaks, and food-borne diseases.<sup>2,3</sup> The 2009 Hajj took place during the recent human pandemic influenza A/H1N1. The first report of human infection with a novel influenza

A/H1N1 virus of swine origin was published in April 2009,<sup>4</sup> but by the time of Hajj, which was in the last week of November, the virus had caused widespread disease worldwide and had been declared as a global pandemic by the World Health Organization on June 11, 2009.<sup>5</sup> Although the case fatality rate outside of Mexico was comparable to that of seasonal influenza, it was much higher in Mexico, and most of the fatalities occurred in individuals <60 years of age.<sup>6,7</sup> In Iran, the first confirmed case was detected in June 2009, and by November 2009, 2662 individuals (1307 women and 1355

men) who had presented in Iran with flu-like illnesses were confirmed to be infected with pandemic influenza A H1N1 virus [A(H1N1)pdm09].<sup>8</sup> Therefore, the spread of the A(H1N1)pdm09 among the susceptible pilgrims during Hajj became a significant possibility. Modern transportation systems, including air travel, allowed many of the infected people, some of whom may have entered during the incubation period, to arrive in Saudi Arabia undetected.<sup>9,10</sup> Such circumstances could have enabled the emergence of the A(H1N1)pdm09 infection among the pilgrims. Hajj-related transmission of A(H1N1)pdm09 by pilgrims returning to their home countries could potentially initiate waves of outbreaks and impose burdens on local healthcare systems. Such health issues could seriously challenge healthcare staff. Memish *et al.*<sup>11</sup> described a consultation between Saudi Arabia and the WHO, which resulted in a plan to decrease the transmission of A(H1N1)pdm09 at the 2009 Hajj. The most important of the recommendations was that the population groups at the highest risk of influenza complications, including pregnant women, patients with chronic diseases, and individuals under 12 years or over 65 years of age, should voluntarily refrain from performing the 2009 Hajj.

Hajj authorities in Iran made the same recommendations to Iranian Hajj participants in 2009. A total of 3200 individuals from the two provinces of Fars and Kohgiluyeh va Buyer Ahmad, southern Iran, participated in the 2009 Hajj. The duration of the Hajj ritual is 1 week but MG lasts for about 30 days.

The present study seeks to determine the rate of infection among the returning pilgrims from Saudi Arabia. To do so, two real-time RT-PCR assays were used to detect RNA of A(H1N1)pdm09 and other seasonal influenza A viruses in collected throat samples from this population. The results could be beneficial in planning and designing the prevention of the virus transmission to the community.

## Methods

### Subject selection

The study was conducted on 305 (9.5%) pilgrims. All 3200 arriving pilgrims were divided into 320 clusters and one person randomly selected from each clusters based on random numbers. One out of each 10 individuals passing through the passport checkout at the arrival gate of Shiraz International Airport was selected with no attention to the presence of clinical influenza-like illness (ILI) symptoms and sent to the sampling division. Posterior pharyngeal swabs were collected from each selected traveler. Therefore, the familial relationship was very weak among those selected. All of the pilgrims selected had arrived in Iran between December 8–11, 2009, and the sampling was con-

ducted for 4 days. A questionnaire containing questions inquiring about any sign or symptom related to respiratory infection during the Hajj was completed for each subject. Although nasopharyngeal swabs could be reportedly more suitable for respiratory virus isolation, both pharyngeal and nasopharyngeal swabs seem to be equally valuable in PCR assays.<sup>12,13</sup> The ethics committee of Shiraz University of Medical Sciences reviewed and approved the study protocol, and written informed consent was obtained from the pilgrims.

### Sample preparation

All the dacron swabs on a flexible plastic shaft were collected in viral transport medium (VTM) and stored at 4°C before being quickly (up to 4 hours) transported to the Professor Alborzi Clinical Microbiology Research Center at Namazi Hospital, Shiraz. The VTM was prepared in-house and contained Hanks BSS with 1% bovine serum albumin, sodium bicarbonate, phenol red, penicillin, and gentamicin. Swab samples were stored at -70°C until RNA extraction.

### RNA extraction

For all samples, nucleic acid was extracted from a 200- $\mu$ l volume of VTM using a commercially available viral RNA isolation kit (High Pure Viral RNA Kit; Roche Diagnostics GmbH, Mannheim, Germany). A standardized amount of internal control RNA, supplied with the real-time PCR kit, was added to the lysis buffer to monitor the efficiency of sample extraction, the elimination of reverse transcription and PCR inhibitors, and the cDNA synthesis process. Negative controls were included in the extraction process between every 20 clinical samples.

### Real-time RT-PCR

A commercially available real-time RT-PCR kit was used to detect and quantify all seasonal influenza A sequences (regardless of serotype), A(H1N1)pdm09 sequences, and the H257Y mutation (oseltamivir-resistant strain) in the neuraminidase sequences of A(H1N1)pdm09 virus-positive samples. All procedures were performed according to the directions and recommendations of the manufacturer's manual (Tamiflu Resistance Genotyping Kit, Mexican Swine Flu Pandemic Strain; PrimerDesign Ltd., Millbrook Technology Campus, Southampton, UK).

The amplification process was performed using TaqMan 1-Step RT-PCR Master Mix Reagents (Roche, Branchburg, NJ, USA) in a 7500 Real-Time PCR System instrument (Applied Biosystems, USA) as follows: 48°C for 30 minute (reverse transcription) and 95°C for 10 minute (DNA polymerase activation), followed by 45 cycles of 94°C for 10 second (denaturation) and 60°C for 60 second (annealing and extension).

## Statistical analysis

Fisher's exact test was used to test the correlations of the age, sex, and each presenting symptom of the subjects with their viral loads. SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical computations. A *P*-value of <0.05 was considered significant.

## Results

We enrolled 305 Hajj pilgrims in the study; this group included 132 (43.3%) men and 173 (56.7%) women, ranging in age from 24 to 65 years, [mean  $\pm$  standard deviation (SD): 49.2  $\pm$  8.8 years]. Based on the collected data from the questionnaires, 147 (48.2%), 141 (46.2%), and 185 (60.6%) were with cough, sore throat, and rhinorrhea or congestion, respectively. However, the severity and duration of the conditions were unknown. Only 10.5% of the cases had fever, sore throat, and cough simultaneously. Two hundred and ninety-eight (97.7%) of the study population have been given influenza vaccine during 2009–2010 season (Table 1).

The real-time PCR result for A(H1N1)pdm09 was positive in five (1.6%) cases, and genomic RNA from other influenza A viruses was detected in eight (2.6%) patients (Table 2). All A(H1N1)pdm09 sequences were sensitive to oseltamivir and lacked the H275Y mutation. All the influenza PCR-positive patients had cough and sore throat, of whom seven patients had fever at the time of sample collection. A total of eight infected individuals with seasonal influenza A viruses had received seasonal influenza vaccine before Hajj.

No correlation was observed between viral load and age or sex. However, a significant correlation was found between viral load and fever (*P* = 0.001).

## Discussion

Mass gatherings like the Hajj could facilitate the emergence of widespread epidemics of infectious diseases.<sup>14</sup> The coincidence that the Hajj MG in 2009 occurred at the same time as the outbreak of A(H1N1)pdm09 might have potentially aggravated the illness among the pilgrims. The concerns about infected pilgrims' arrivals to their home countries after having participated in the Hajj could and spread the disease have triggered new waves of the flu infection. For these reasons, the Hajj authorities convened a large international consultation meeting prior to Hajj to provide local health authorities and international health community with evidence-based guidelines to prevent disease transmission among pilgrims in Saudi Arabia and their countries of origin on their return. The health ministry in Iran followed these recommendations inclusive of not allowing high-risk groups to leave Iran for the Hajj.

**Table 1.** Characteristics and symptoms of the 305 Hajj pilgrims

Characteristics	Value
Female sex – no./total no. (%)	173/305 (56.7)
Age	
Mean $\pm$ Std. Deviation – year	49.2 $\pm$ 8.82
Range – year	41
Clinical sign – no./total no. (%)	
Fever	34/305 (11.1)
Cough	147/305 (48.2)
Sore throat	141/305 (46.2)
Nasal symptoms	185/305 (60.65)
Myalgia	97/305 (31.8)
Headache	126/305 (41.3)
Diarrhea	5/305 (1.6)
Vomiting	5/305 (1.6)
Vaccinated with influenza vaccine during 2009–2010 season – no./total no. (%)	298/305 (97.7)

The present study aimed to determine the point prevalence of A(H1N1)pdm09 among the returning pilgrims in Iran. Real-time PCR revealed that of the 305 pilgrims sampled, A(H1N1)pdm09 was detected in five (1.6%) and other influenza A viruses in eight (2.6%) cases. As the results indicate, there was a low point prevalence of A(H1N1)pdm09 shown. The reasons could be the virulence of the A(H1N1)pdm09 not being higher than that of seasonal one and also the instructions given to the pilgrims prior to the departure about respiratory etiquette, mask use, and hand hygiene and making them aware of the risk factors associated with seasonal and pandemic influenza. It is noteworthy that 298 (97.7%) of the pilgrims had been vaccinated against the 2009–2010 seasonal flu viruses before leaving Iran for Saudi Arabia. At the time of the Hajj, the A(H1N1)pdm09 vaccine was under great demand with significant shortage of supplies across the globe,<sup>15</sup> and none of these pilgrims had received the pandemic flu vaccine before or during the Hajj. Despite the seasonal influenza vaccination, eight cases of influenza A were detected in pilgrims returning to Iran, which might have been because of poor vaccine efficacy as previously reported.<sup>2</sup> The results also showed that many of the pilgrims had experienced respiratory signs and 147 (48%) and 141 (46%) had developed cough and sore throat, respectively.

The presence of fever in acute respiratory infection is indicative of the severity of the infection. Of the studied cases, 10.5% had fever, cough, and sore throats during their travel, but none were hospitalized. As revealed in the present study, the load of influenza A and the presence of fever was positively correlated, that is, the higher the titer of the virus, the greater probability of the fever. The number of

**Table 2.** Laboratory information and presenting symptoms of A(H1N1)pdm09 and seasonal influenza A viruses-infected patients

Patients No.	Age (year)	Sex*	PCR result	Quantity†	Clinical sign					2009–2010 influenza Seasonal vaccination
					Fever	Cough	Sore Throat	Vomiting	Diarrhea	
29	41	F	OIA	905 821	+	+	+	–	–	+
96	50	F	pH1N1	1025	–	+	+	–	–	+
117	64	F	OIA	1105	–	+	+	–	–	+
126	53	F	pH1N1	137 929	+	+	+	–	–	+
135	46	F	pH1N1	1713	–	+	+	–	–	+
150	45	F	OIA	106 006	+	+	+	–	–	+
195	51	M	pH1N1	27 604	+	+	+	–	–	+
199	50	M	OIA	144 824	+	+	+	–	–	+
224	38	M	OIA	2340	+	+	+	–	–	+
244	51	M	OIA	46 211	+	+	+	+	+	+
273	40	F	OIA	975	–	+	+	–	–	+
290	45	F	pH1N1	996	–	+	+	–	–	+
305	53	F	OIA	1639	–	+	+	–	–	+

pH1N1, pandemic influenza A/H1N1 2009; OIA, Other Influenza A.

\*F = Female, M = Male.

†Copy per 10 µl of viral transport media.

cases with upper respiratory symptoms including cough and sore throat along with fever (among a few of the cases) was rather high.

Asymptomatic and prolonged asymptomatic shedding are associated with Influenza A, which may escape a patient's notice and spread to others who are in contact with those infected. This is an important point which deserves careful consideration on the part of health staff. Although A(H1N1)pdm09 and other influenza A viruses were detected in only five (1.6%) and eight (2.6%) of the pilgrims who were tested at the airport, respectively, some might have been infected and recovered already during the 30 day Hajj. Therefore, although A(H1N1)pdm09 infection had been circulating during the Hajj MG, other respiratory tract infections might have been circulating besides the various influenzas. Similar findings were also reported on overseas travelers, in which despite the A(H1N1)pdm09, rhinovirus and other influenza viruses were also frequent causes of ILI.<sup>16</sup>

Given that the first cases of A(H1N1)pdm09 infection in Iran had been reported in June 2009, it seems that 6 months is a sufficient interval for a newly emerged influenza virus to spread among a susceptible population, especially in the presence of close social contact patterns, which have been previously reported to enhance the rapid spread of the virus.<sup>17</sup> Therefore, it can be concluded that a number of Iranian pilgrimage candidates had antibodies against the virus because of natural A(H1N1)pdm09 infection prior to the Hajj season. The results do not indicate any severe complication of A(H1N1)pdm09 infection during the Hajj. Nevertheless, susceptible pilgrims may be at risk

of infection during this 1-month stay in Saudi Arabia under Hajj MG conditions.<sup>15</sup>

The present findings, in agreement with recent studies,<sup>18,19</sup> suggest that A(H1N1)pdm09 infection during the Hajj was not associated with mortality or severe disease requiring hospitalization. This finding is understandable in view of the fact that there were no members of high-risk groups such as pregnant women or individuals with chronic health conditions among the pilgrims, and secondly, as previous reports indicate, A(H1N1)pdm09 infection has not been associated with high mortality rates and finally the instructions given to the pilgrims about contact and hand hygiene and respiratory etiquette.<sup>20–22</sup> Border screening can serve as an important method of controlling and preventing epidemic infection from entering a new place.<sup>23,24</sup> In a recent study, Infrared Thermal Image Scanners (ITIS) were used to screen febrile travelers during a seasonal epidemic of predominant influenza B. It was shown that many of the influenza B sufferers were without fever; therefore, in that case, ITIS could not serve as an effective method for identifying Influenza B cases. However, during epidemics of Influenza A, where the majority of affected people develop a fever, ITIS can serve as an effective screening tool.<sup>25</sup> The border screening performed in the present study revealed that the arrival of pilgrims from the Hajj to their home countries did not cause a new wave of A(H1N1)pdm09 infection in the community. This finding can help avoid the overestimation of the potential threat of infection and the improper mobilization of social and healthcare services.

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## Conflicts of interest

The authors state that they have no conflicts of interest to declare.

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