Influenza viral infections among the Iranian Hajj pilgrims returning to Shiraz, Fars province, Iran

Afagh Moattari,^a Amir Emami,^a Mohsen Moghadami,^b Behnam Honarvar^b

^aDepartment of Bacteriology and Virology, Shiraz University of Medical Sciences, Shiraz, Iran. ^bHealth Policy Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

Correspondence: Afagh Moattari, Department of Bacteriology & Virology, Shiraz Medical School, Zand Street, Setad SQ, Shiraz City, Fars province, Iran. E-mail: moattaria@yahoo.com

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Background Annually over two million Muslims from across the world converge on Mecca to perform the Hajj pilgrimage. Overcrowding at the Hajj facilitates spread of communicable diseases, especially respiratory infections. The aim of this study was to determine the attack rate of seasonal and pandemic influenza among returning Iranian pilgrims after the 2009 Hajj.

Methods Clinical data and throat swabs were collected at Shiraz airport from symptomatic Iranian pilgrims of Fars province who were returning from the Hajj between 15 and 21 December 2009. The specimens were tested at the Shiraz University of Medical Sciences Influenza Research Center for influenza viruses by cell culture and real-time reverse transcriptase polymerase chain reaction (RT-PCR) according to standard protocol.

Findings Out of 3000 pilgrims from Fars province who attended the Hajj 2009, 275 symptomatic pilgrims were recruited into this study. Pilgrims had fever, cough, muscle ache and sore throat in various combinations. Twenty-five (9.1%) pilgrims had influenza by virus culture and these were as follows: influenza B ($n = 17$), influenza A H3N2 ($n = 8$) and pandemic H1N1 ($n = 5$), whereas 33 (12%) had influenza by RT-PCR: influenza B ($n = 20$), influenza A H3N2 ($n = 8$) and pandemic H1N1 ($n = 5$).

Interpretation Both seasonal and pandemic influenza infections occurred among the Iranian Hajj pilgrims; seasonal viruses were more common than the pandemic viruses even though all pilgrims were vaccinated against seasonal influenza.

Keywords Hajj, Iran, pandemic influenza, pilgrims.

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Introduction

Following the first report of H1N1 pandemic influenza 2009 (pH1N1) in Mexico, human-to-human transmission of the virus resulted in rapid global spread reaching, within weeks, as far as Spain, UK, Africa, Asia, and Australia.^{1–3} It was speculated that high population density and a mass gathering that took place in Iztapalapa, Mexico, during the Easter season of 2009, contributed to the rapid spread of the infection.⁴ Annually more than two million Muslims, including one hundred and thousands from Iran, converge on Mecca, Saudi Arabia, to perform the Hajj pilgrimage, one of the world's largest mass gatherings. The incredibly large and dense crowding of people facilitates the spread of communicable diseases especially respiratory infections. Several studies have reported a high attack rate of seasonal influenza among the Hajj pilgrims including those from Iran.^{5–7} The Hajj 2009 took place amidst fear of the H1N1 pandemic in late November the same year. A few studies have been published on the epidemiology of pH1N1 at the Hajj among Egyptian and international pilgrims showing a low prevalence of the disease, but the studies were constrained by limitations as

acknowledged by the authors,^{8,9} and the epidemiology of pH1N1 among Iranian pilgrims has not yet been reported.

The aim of this study was to determine the attack rate of seasonal influenza and pH1N1 among the Iranian Hajj pilgrims returning to Shiraz, the principal city of Fars province of Iran.

Methods

Setting and participants

The target population was 3000 returning Iranian pilgrims of Fars province. Pilgrims with symptoms of fever, cough and sore throat were recruited at Shiraz airport between 15 and 21 December 2009. After briefing the study and obtaining verbal consent, research physicians collected demographic and clinical data in a standard questionnaire and obtained 275 throat swabs from the pilgrims (one swab from each subject). The specimens were placed in viral transport media and transported, under refrigeration, to the virology laboratory of Shiraz University of Medical School (SUMS) and stored at -70°C until tested. The study was approved by the Ethics Committee of SUMS dated 2 December 2009.

Virus isolation

Influenza A and B virus reference strains were obtained from the National Institute for Biological Standards and Control (NIBSC), UK. The swabs were then vortexed in 5 ml medium for a few minutes to dislodge and suspend adherent viruses. The Madin-Darby canine kidney cell confluent monolayers were inoculated with 200 microliters of the viral suspension. The monolayers were maintained in serum free Dulbecco's Modified Eagle's Medium (Sigma Aldrich, Munich, Germany) supplemented with 2 mg/ml trypsin (Gibco BRL; Life Technologies, Gibco, Australia) 100 µg/ml streptomycin and 100 units/ml penicillin G. The cultures were incubated at 34°C and examined daily for cytopathic effect that was confirmed by the ability of infected cultures to agglutinate guinea pig erythrocytes no later than 7 days postinfection.¹⁰

RNA extraction and RT-PCR

RNA extraction was carried out using Roche High Pure Viral RNA Extraction Kit (Roche, Berlin, Germany) according to the manufacturer's instructions. Extracted RNAs were kept at -80°C until further processing. RT-PCR was carried out using SuperScript III Platinum One-Step Quantitative RT-PCR kit manufactured by Invitrogen (Carlsbad, CA, USA). Real-time runs were performed on the Corbett 6000 Rotor-Gene system (Corbett, Victoria, Australia). The reaction comprised 4 µl of extracted RNA combined with 16 µl of master mix including 2X reaction mix, SuperScript III RT/Platinum Taq Mix, 5.4 µl RNase-DNase free water, and 0.4 µl of each primer and probe with the concentration of 40 µl and 10 µl, respectively. Each sample of RNA was tested by separate primer/probe sets for the detection of influenza universal swine (swFLUA), swine H1 and RNase P. According to the CDC real-time RT-PCR protocol (2009), the cycling conditions included a 30 min RT step at 50°C, followed by enzyme inactivation at 95°C for 2 min and the PCR step comprised 45 cycles at 95°C for 15 s, 55°C for 30 s, and 72°C for 30 s.¹¹ Data acquisition and analysis of the real-time PCR assay were accomplished using the Rotor-Gene data analysis Software, Version 6.0A (Corbett).

Statistical analysis

The chi-square test was used for comparing categorical variables, and a *P* value <0.05 was considered significant.

Results

Of 275 recruits, just over half (50.9%) were men. Their median age was 46 (range 20–70 years); about 45% were older than 50 years (Figure 1). All pilgrims received seasonal influenza vaccine 2 weeks before departure, but none received pH1N1 vaccine. All pilgrims complained of fever,

Figure 1 Age distribution of Hajj pilgrims in Fars province of Iran.

and along with this, cough, muscle ache and sore throat in various combinations (Table 1). Overall 25 (9.1%) patients were identified to have influenza by virus culture and 33 (12%) had influenza by RT-PCR; there was no significant difference between the diagnostic yields of the two methods (Table 2). Viruses isolated by culture included influenza B 17 (6.2%), influenza A H3N2 5 (1.8%) and pH1N1 3 (1.1%). Viruses detected by RT-PCR were influenza B 20 (7.3%), influenza A H3N2 8 (2.9%) and pH1N1 5 (1.8%).

Discussion

In this study, we have demonstrated that both seasonal influenza and pH1N1 are important in the Hajj pilgrims. It is a concern that despite being vaccinated, a considerable proportion of pilgrims had seasonal influenza. The attack rate of seasonal influenza of 10.2% (28/275) in our study is similar to that of most other studies conducted in previous years involving, for instance, pilgrims from Iran, UK and Saudi Arabia,^{5,7} but higher than the prevalence of seasonal influenza reported by employees of the Saudi Government for the 2009 Hajj.⁸ Memish and his colleagues have claimed that only seven recruits (five with non-pandemic influenza A and two with influenza B) out of a total 3218 pilgrims had seasonal influenza that translates to a prevalence rate of 0.2%. The authors, however, have failed to explain the low prevalence of seasonal influenza in their study.⁸ In a separate study, the authors have further claimed that none of the 184

Table 1. Distribution of symptoms among the Hajj pilgrims in Fars Province of Iran

Symptoms	Number
Fever	275
Sore throat and fever	200
Myalgia and fever	220
Cough and fever	150

Table 2. Distribution of influenza viruses using culture and real-time PCR in pharyngeal secretions of the Iranian Hajj pilgrims in 2009 ($n = 275$)

Virus	Culture	Real time PCR
Influenza B	17 (6.2%)	20 (7.3%)
Influenza A (H3N2)	5 (1.8%)	8 (2.9%)
Pandemic influenza new (H1N1)	3 (1.1%)	5 (1.8%)

healthcare workers they tested before and after the Hajj had influenza.¹² Interestingly, pH1N1 was not the dominant strain among Iranian pilgrims and was reported in only 1.8% pilgrims even though they were unvaccinated. This compares with 0% prevalence rate in fully vaccinated Egyptian pilgrims,⁹ and 0.2% in arriving and 0.1% in departing international pilgrims of whom 39% and 29%, respectively, were vaccinated.⁸ Apart from the differences in study designs, the discrepancies may have stemmed from vaccination status of the pilgrims or pre-existing immunity. The latter may be important because about 45% pilgrims in our study were aged >50 years (Figure 1) and therefore likely to have been exposed to H1N1 before 1957.¹³ It is also possible that many non-susceptible pilgrims may have had symptomatic or non-symptomatic influenza before traveling to the Hajj, hence accounting for the low attack rate of pH1N1 in this and other studies. It is also interesting to note that the extremely low burden of pH1N1 with a high case-fatality ratio (of 5%) reported by Saudi authorities has sparked controversy.¹⁴ Most of the published studies including ours may have underestimated the actual burden of influenza as the recruitment occurred after the conclusion, or before the start of the Hajj.^{8,9,12} Real-time surveillance during the peak period of the Hajj would have revealed the true picture. A recent paper reports that 22% of intensive care admissions at hospitals in the Hajj sites were because of PCR-confirmed pH1N1.¹⁵

The fact that despite receiving seasonal influenza vaccine a substantial proportion of pilgrims had influenza is not surprising as other reports suggest the non-significant role of the vaccine against influenza at the Hajj.^{6,7,16} It is important to explore the role of other measures, for example, surgical masks, in preventing influenza at the Hajj.¹⁷

Conclusion

Both seasonal and pandemic influenza are important in the Hajj pilgrims. During the pandemic year, despite vaccination, seasonal influenza affected one tenth of the symptomatic Iranian pilgrims. The role of other preventive measures against influenza need to be explored and enhanced surveillance of influenza should continue.

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