



## Trends in surveillance data of influenza virus in Tehran before decreasing dispatch of Iranian Hajj pilgrims to Mecca

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### Abstract

**Background:** Respiratory infections, especially viral infections, are the most prevalent infection affecting Hajj pilgrims. Commonly 3 major human influenza viruses (A/H1N1, A/H3N2, and B) are responsible for these morbidities. The present study was conducted to develop a statistical report on human influenza in Hajj pilgrims.

**Methods:** Nasal and throat samples were collected from 232 returning Iranian pilgrims in hospitals of IUMS. All samples were kept in the refrigerator at 4 °C and stored at -70 °C until RNA extraction. RNA extraction was performed by QIAamp viral RNAmini kits (QIAGEN, Hilden, Germany) and influenza viruses were detected by TaqMan RT-PCR.

**Results:** Participants included 115 (49.5%) male and 117 (50.5%) female patients, with the age range of 10 to 93 years (mean: 53 years). The pandemic and seasonal influenza A (H1N1) virus were detected in 2 (0.8%) and 20 (8.6%) pilgrims, respectively, and also influenza B was identified in 1 person (0.4%).

**Conclusions:** Since the probability of an influenza pandemic has been anticipated for the coming years, it seems necessary to plan a continuous monitoring of large gatherings like Hajj and conduct statistical studies in the region. Moreover, material surveillance in humans needs to be boosted. Therefore, results of influenza research can be important for developing WHO reports.

**Keywords:** Influenza, Hajj, Pilgrim, Iran

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### Introduction

Influenza A is an enveloped negative-strand RNA virus with 8 segmented genomes, which are classified as belonging to the orthomyxovirus family (1-3). This virus is a circulating pathogen that causes morbidity and mortality worldwide among humans (4, 5). Based on surface glycoproteins, this virus is divided into 6 genera, of which 3 affect humans: Influenza virus A, B, and C (1, 6-8). The diagnostic tests for this virus, which include viral isolation, biopsy or autopsy tissue section, rapid diagnostic tests, se-

rologic methods, and confirmation tests, include immuno-histochemical techniques or in situ hybridization (9). Haemagglutination test is used to quantify the titer of influenza virus and it is based on binding of the HA antigen with the sialylated glycans of erythrocytes.

Spread of communicable diseases, especially respiratory infections, is facilitated in large and dense crowds. Annually, more than 3 million pilgrims from > 160 countries, including thousands from Iran, gather in Saudi Arabia to perform Hajj, which is one of the largest gatherings in the

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#### ↑What is “already known” in this topic:

Outbreaks of influenza viruses that spread via respiratory route are common among Hajj pilgrims. Since a few years ago, pilgrims have been recommended to take influenza vaccine before dispatch to Mecca. Few studies in Iran reported different information that showed vaccination coverage has been satisfying in recent years.

#### →What this article adds:

The present study investigated the prevalence of influenza-like illness (ILI) in Hajj pilgrims after returning to Tehran. So, surveillance data of influenza virus in Tehran was examined to report the rate of ILI against influenza vaccine uptake among Iranian Hajj pilgrims over the last 2 years.

Table 1. Primers and probes sequence used in this study

Primers & probes	Position (bp)	Sequences (5' > 3')	Targeted genes
Flu A(H1N1)forward	393–416	TGAGATATTCCTCAAGACAAGTTC	Hemagglutinin
Flu A(H1N1) reverse	489–467	TTTGTAAGAAGCTTTTGCTCCAG	
Flu A(H1N1) probe	426–451	TCATGACTCGAACAAGGTGTAACGG	Hemagglutinin
Flu A(H3N2)forward	744–770	CTATTGGACAATAGTAAACCGGGRGA	
Flu A(H3N2)reverse	898–921	GTCATTGGGRATGCTTCCATTGG	
Flu A(H3N2) probe	799–821	AAGTAACCCCKAGGAGCAATTAG	Nonstructural (NS)
Flu B forward primer	43–59	GGAGCAACCAATGCCAC	
Flu B reverse primer	138–147	GTTTAGCGGTCTTGACCAG	
Flu B probe	61–80	ATAAACTTTGAAGCAGGAAT	Matrix
Flu A forward	1393–1416	CCGAGGTCGAAACGTATGTTCTCTCTATC	
Flu A reverse	1567–1589	TGACAGAATTGGTCTGTCTTTAGCCATTCCA	
Flu A probe	1424–1451	ATCTCGGCTTTGAGGGGGCCTG	

world. The pilgrims move from one place to another in Mecca to complete Hajj ritual (10). Thus, this gathering can increase the risk of public health issues, such as food-borne diseases and respiratory infections, particularly of the pandemic influenza A/H1N1, A/H3N2, and B. A high rate of attack of seasonal influenza has been reported among the Iranian Hajj pilgrims (11). A few studies have reported an epidemiology of influenza A (H1N1, H3N2) and B infection among the Hajj pilgrims. The first aim of this study was to provide a statistical report of human influenza A viruses (H1N1, H3N2) and influenza B viruses of Iranian pilgrims in Hajj in hospitals of Iran University of Medical Sciences (IUMS) during 2013 and 2015. The second aim was to assess pilgrim's beliefs and intentions on taking the vaccine to prevent influenza during Hajj.

## Methods

### Patients' enrolment and specimen preparation

This was a cross-sectional study and the target population was 232 returning Iranian pilgrims during 2013, 2014, and 2015 in Tehran province. Pilgrims had acute respiratory infection (ARI) and symptoms including fever, cough, and sore throat. The protocol was approved by the ethical research committees at IUMS.

In the study, patients were interviewed by investigators who used a standardised questionnaire and filled out a form about medical history, clinical data, and demographic characteristics. The interviewer described the voluntary nature of participation and provided information on antiviral drugs and influenza vaccine.

All specimens were taken from nose and throat by Dacron swab and collected into sampling media (Hank's Balanced Salt Solution) at the laboratory, then, they were kept in the refrigerator at 4 °C and stored at –70 °C until RNA extraction.

### RNA extraction

RNA extraction was done by QIAamp viral RNAmini kits (QIAGEN, Hilden, Germany), according to the kit instructions, and all extracts were stored at –80 °C. Primers

and probes were designed according to available sequences in the Gene Bank database of the National Centre for Biotechnology Information (NCBI) using Primer3plus (<http://primer3plus.com/>).

### RT-PCR

The amplification process was done by TaqMan RT-PCR master mix ReagentsQIAamp viral RNAmini kits (QIAGEN, Hilden, Germany), and tests were conducted with Corbett 6000 Rotor-Gene system (Corbett, Victoria, Australia). Each reaction included 4 µL of extracted RNA mixed with 16 µL of master mix, which included 2xRT-PCR Quick Master Mix, nuclease-free water, control primer forward and reverse, and 50 mM Mn (OAc)<sub>2</sub>. All samples were tested by separated primer and probe sets to identify H1N1 and H3N2 (Table 1). According to CDC protocol, RT-PCR cycling included 42 °C for 30 minutes, 7 minutes for Taq activation at 95 °C, then, 95 °C for 15 seconds, and an annealing extension step with temperatures, ranging from 50 to 65 °C for 30 seconds for 45 cycles.

### Ethics

This research was approved by Ethics Committee of Iran University of Medical Sciences (IUMS) (project number: 93-01-30-24468).

### Statistical analysis

In this study, we used Fisher's exact test to determine the differences in proportions. Statistical computations were performed by SPSS 18.0 for Windows, GraphPad 5, and Excel, and p-value <0.05 was considered significant.

## Results

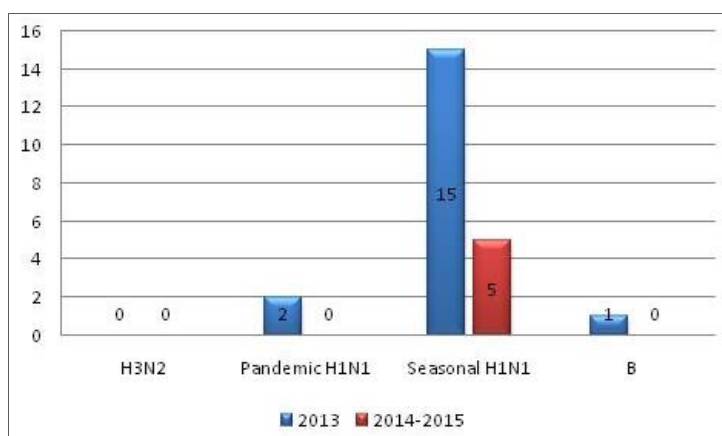
We enrolled 232 Iranian pilgrims in this study during 2013 to 2015, of them 115 (49.5%) were male and 117 (50.5%) female, and their age ranged from 10 and 93 years (mean: 53 years). The prevalence of seasonal H1N1 influenza (18 persons; 14.1%) was significantly more (p=0.02). In 2013 compared to 2014 (5 persons; 4.8%). Detailed description of characterization and comparison of these years is presented in Tables 2 and 3 and Fig. 1. Data analysis

Table 2. Distribution subtypes of influenza in 2013 and 2014–2015

Influenza subtype	Year 2013		Year 2014–2015		p
	Positive	Negative	Positive	Negative	
A/H3N2	0	128	0	104	1.01
Seasonal H1N1	15	113	5	99	0.06
Pandemic H1N1	2	126	0	104	0.07
Flu B	1	127	0	104	0.08

**Table 3.** Laboratory and symptoms data in Hajj pilgrims positive patients (n=23)

Sex	Mean of Age	n	H1N1 Pandemic	H1N1 Seasonal	H3N2	Flu B	Fever	Cough	Myalgia
Male	63.00	11	1	10	0	0	8	8	5
Female	46.41	12	1	10	0	1	7	8	11
Total (%)	54.34	23(100)	2(8.7)	20(86.9)	0(0)	1(4.3)	15(65.2)	16(69.6)	16(69.6)

**Fig. 1.** Influenza distribution in Hajj pilgrims in Tehran in 2013 and 2014–2015

showed that 11 of 115 males and 12 of 117 females were positive, of which 2, 20, and 1 had pandemic H1N1, seasonal H1N1, and influenza B, respectively. Also, results indicated that the mean age of influenza positive and negative patients was 53.59 and 54.34 years, respectively. Seasonal influenza vaccination coverage for the preceding Iranian Hajj Pilgrims was 100%, moreover, influenza vaccine is administered to all the Hajj Pilgrims before travelling in the Islamic Republic of Iran.

### Discussion

Upper respiratory tract (URT) infection is a major health problem during Hajj. The exact prevalence of URT infection among pilgrims is not known. This infection was estimated to range 20% to 80% depending on method of detection (12). One of the most important global causes of morbidity and mortality is influenza, resulting in an estimated 3 to 5 million cases of severe influenza illness and 250 000 to 500 000 deaths worldwide annually. Past studies have demonstrated that influenza viruses are the most common viral infection among Hajj pilgrims and vaccination cannot efficiently prevent this infection. Over more than 2 consecutive years, we conducted a cross-sectional study of influenza in respiratory specimens collected from Iranian Hajj pilgrims after leaving Saudi Arabia for Tehran. Our study provided a new insight into the pandemic (H1N1) influenza and influenza virus A(H3N2) in Tehran. Also, in this study, we checked both seasonal and pandemic H1N1, H3N2, and influenza B viruses in Hajj pilgrims. Rate of pH1N1 was very low among pilgrims (about 0.86% ( $p>0.05$ )), seasonal H1N1 was 8.6% ( $p=0.06$ ), H3N2 0% ( $p>0.05$ ), and influenza B 0.43% ( $p>0.05$ ). Prevalence of all influenza subtypes was higher in 2013 than in 2014 and 2015 ( $p>0.02$ ). Reduction in the prevalence of these influenza subtypes in 2014 compared to 2013 can be due to the increase in levels of personal hygiene or prevention methods, such as vaccination. The attack rate of seasonal influenza in our study

was 8.6% (20/232), which is similar to previous studies conducted in Iran, the UK, and Saudi Arabia (5). Due to special circumstances in Hajj, such as large number of pilgrims in a small area, it is not easy to prevent the transmission of respiratory infections, including influenza.

Moattari et al. in Shiraz (2012) reported the prevalence of influenza in pilgrims. Using virus culture, we found that 25 (9.1%) pilgrims had influenza: influenza B ( $n=17$ ), influenza A (H3N2) ( $n=8$ ), and pandemic H1N1 ( $n=5$ ). Moreover, using RT-PCR method, we also found that 33 (12%) pilgrims had influenza: influenza B ( $n=20$ ), influenza A (H3N2) ( $n=8$ ), and pandemic H1N1 ( $n=5$ ) (11). In a study by Moattari et al., the attack rate of seasonal influenza was 10.2%, and this result was similar to that of other studies conducted in previous years in the UK, Iran, and Saudi Arabia (11).

Another study done by Rashid et al. (2005) reported the rate of influenza virus in UK pilgrims as follow: Influenza A (H3) was 54% of the virus-positive samples, influenza B 19%, and influenza A (H1) 3% (12). In another study, rate of viral respiratory infections was compared between Saudi and UK pilgrims by Rashid et al. Results of this study were as follow: rate of influenza virus infection in both geographical regions was similar (10%) (13). Memish et al. (2009) reported that the prevalence of influenza A virus was 0.2% in Saudi Arabia (14).

In 2009, Ziyaeyan et al., by PCR, reported the incidence of acquired H1N1 to be 1.6% among returning Iranian pilgrims during the Hajj season (15). The difference in prevalence can be due to increased levels of personal hygiene or prevention methods such as vaccination.

Data analysis revealed that the rate of influenza increased in 2013 compared to 2014 and 2015, which is one of the reasons for the reduced deployment in Hajj due to political problems.

Finally, the results of this study and similar studies could improve hygiene levels and reduce disease and mortality

during Hajj.

Results of researches on influenza can be important for developing WHO reports, but there is still a need for support and enhancement. To improve systematic surveillance in humans, surveillance data should be presented not only within each country but also internationally.

### Conclusion

Study of the frequency of seasonal and pandemic influenza in Hajj pilgrims is necessary for annual monitoring. Despite vaccination, seasonal influenza has affected many of the symptomatic Iranian pilgrims. However, other effective factors in the prevention of influenza disease should not be ignored. On the other hand, due to political reasons, pilgrims dispatching to Mecca was decreased in Iran, so the preliminary results of the present study cannot be extrapolated to all pilgrims because this work was part of a large-scale study that should be continued in the following years.

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### Conflict of Interests

The authors declare that they have no competing interests.

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