Original Article

Demographic distribution and transmission potential of influenza A and 2009 pandemic influenza A H1N1 in pilgrims

Ahmed Ashshi[1], Esam Azhar[2,3], Ayman Johargy[4], Atif Asghar[4], Aiman Momenah[4], Abdulhafeez Turkestani[5], Saad Alghamdi[1], Ziad Memish[6], Ahmed Al-Ghamdi[3], Maha Alawi[7], Sherif El-Kafrawy[3], Mohomed Farouk[4], Steve Harakeh[3], Taha Kumosani[8], Hatim Makhdoum[9], Elie K Barbour[8,10]

[1] Department of Laboratory Medicine, Faculty of Applied Medical Science, Umm Al-Qura University, Makkah, Saudi Arabia
[2] Department of Medical Laboratory Technology, Faculty of Applied Medical Science, King Abdulaziz University, Jeddah, Saudi Arabia
[3] Special Infectious Agents Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia
[4] Department of Microbiology, Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia
[5] Preventive Medicine, Health Directorate, Makkah region, Ministry of Health, Saudi Arabia
[6] Preventive Medicine Directorate, Ministry of Health, Riyadh, Saudi Arabia
[7] Infection Control Unit, King Abdulaziz University Hospital, Jeddah, Saudi Arabia
[8] Biochemistry Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
[9] Virology Unit, Jeddah Regional Laboratory, Ministry of Health, Saudi Arabia
[10] Department of Animal and Veterinary Sciences, Faculty of Agricultural and Food Sciences, American University of Beirut, Beirut, Lebanon

Abstract

Introduction: The World Health Organization's persistent reporting of global outbreaks of influenza A viruses, including the 2009 pandemic swine A H1N1 strain (H1N1pdm09), justified the targeted surveillance of pilgrims during their annual congregation that pools more than two million people from around 165 nations in a confined area of Makkah city in the Kingdom of Saudi Arabia (KSA).

Methodology: A total of 1,600 pilgrims were included in the targeted surveillance of influenza A and the 2009 pandemic swine H1N1 strain in the Hajj (pilgrimage) season of 2010. Each pilgrim responded to a demographic and health questionnaire. Collected oropharyngeal swabs were analyzed by real-time PCR for influenza A viruses, and positive samples were further analyzed for the presence of H1N1pdm09. Fisher's exact test was applied in the analysis of the significance of the distribution of influenza-positive pilgrims according to demographic characters.

Results: A total of 120 pilgrims (7.5%) tested positive for influenza A viruses by real-time PCR. Nine out of the 120 influenza-A-positive pilgrims (7.5%) were positive for H1N1pdm09. Demographics played a significant role in those pilgrims who tested positive for influenza A.

Conclusions: The detection of H1N1pdm09 in pilgrims at their port of entry to the KSA was alarming, due to the high potential of trans-boundary transmission. This situation necessitates the implementation of specific prevention and control programs to limit infection by influenza A viruses.

Key words: demographic distribution; Makkah pilgrims; influenza A H1N1.

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Introduction

Infectious disease are reported to be transmitted faster in crowded areas such as schools [1,2], military environments [3], healthcare facilities [4], international conferences, and airplanes [5,6]. Hajj (pilgrimage) to the holy places of Makkah and Madinah is one of the most crowded mass gatherings in the world, where more than two million people convene in the confined area of Makkah in a short period of time. The current fast global transportation systems, carrying people from these confined areas to other parts of the world, play a significant role in the spread of infections [7,8]. A good example was the fast transmission of H1N1pdm09 around the globe in a short period of six weeks, after it originated in Mexico in April 2009 [9].

The antigenic and genetic characteristics of the 2009 H1N1 strain of swine origin have been documented [10]. This strain was rapidly transmitted globally, resulting in a declaration of a phase 6 pandemic alertness by the World Health Organization (WHO). Unfortunately, after more than three years, the same H1N1pdm09 re-emerged in the West Bank area of the Middle East, causing the death of nine individuals until the end of January 2013 [11]. In addition, this same strain of pandemic H1N1 is nowadays rated as the second in rank, after the H3N2 strain, in causing outbreaks in humans all over the world, including Europe (Denmark and France) and Africa (Democratic Republic of Congo) [11].

This alarming situation resulted in the preparation and implementation of surveillance programs around the globe for re-emerging outbreaks by influenza A viruses, including H1N1pdm09 [11]. The pilgrimage to Makkah city in KSA annually attracts more than two million people from about 165 countries around the globe, to a confined area of about 356,800 m^2, resulting in overcrowded conditions that last for a period of 10 days. The nature of such a pilgrimage allows for pooling of human etiologic agents, originating from different areas of the world, in one confined area; this drove the KSA to establish public health security measures in response to the H1N1pdm09 global outbreaks [12].

The literature showed the effect of certain demographic variables on the frequency of influenza infections in populations from different regions around the world [13-15]. The demographic variables that could affect the distribution of influenza A infections in populations are gender [16-19], age [15], clinical signs [20], vaccination against flu [21], usage of antimicrobials [22,24], smoking habits [25,29], and ethnicity [30,31].

The objective of this research was to present a demographic distribution of influenza A and H1N1pdm09 among pilgrims harboring the virus who were surveyed upon their arrival to the first port of entry at King Abdulaziz International Airport, Jeddah city, KSA.

Methodology

Questionnaire and sampling of pilgrims

Permission to present the questionnaire and to collect oropharyngeal swabs from pilgrims was obtained from the local authorities at King Abdulaziz International Airport in Jeddah city, KSA. Ethical approval was obtained from the ethical committee of King Abdulaziz University (code 010-CEGMR-01-ETH-RESTRICTED). An informed consent form was signed by those who agreed to participate. The questionnaire included the following demographic data: nationality, gender, age, flu vaccination status, clinical signs of sore throat and coughing, antimicrobial use, and smoking habits. A total of 1,600 pilgrims were included in this study in the Hajj (pilgrimage) season of 2010. An oropharyngeal swab was collected from each participant, stored at 4°C in a sterile viral transport medium, and sent to the laboratory for analysis using real-time PCR. No attempts were made to isolate the virus using tissue culture-based techniques.

Real-time PCR analysis

The Real-Time PCR Ready Kit (Roche, Bassel, Switzerland) was used according to the manufacturer's instructions. The manufacturer's method was adopted from the Centers for Disease Control and Prevention (CDC)'s procedure, recommended for laboratories around the world for detection of influenza A and H1N1pdm09 viruses [32]. Briefly, the Roche Ready Kit included primers and probes specific for the conserved gene of the matrix protein 2 (M2) of the influenza A viruses. In addition, the kit had primers and probes specific for the detection of H1N1pdm09 [32]; the kit excludes influenza infections other than influenza A and provides specific detection for H1N1pdm09. The kit provided positive controls for influenza A and the H1N1pdm09 viruses. A volume of 5 µL of the extracted viral RNA was added to 15 µL of the provided PCR mix in the reaction capillary. The samples and controls were centrifuged for 15 seconds at 400 g, and the liquid portion at the bottom of the capillaries was collected. The amplification and detection were performed in a Light Cycler 2.0 (Roche, Mannheim, Germany). The following PCR conditions were used: reverse transcription (1 cycle) at 55°C for 8 minutes, initial denaturation (1 cycle) at 95°C for 30 seconds, amplification (45 cycles) with denaturation at 95°C for 1 second, annealing at 60°C for 20 seconds, extension at 72°C for 1 second, and cooling at 40°C for 30 seconds.

Statistical analysis

Statistical analysis was performed using Fisher's exact test [33]. This test was used to examine the distribution of the virus among the different demographics of the pilgrims and to determine the frequency of the virus in pilgrims according to their nationalities. Significant differences were reported at P values of < 0.01 or < 0.05.

Results

Distribution of influenza-A-positive pilgrims according to nationality

The prevalence of influenza A among the participants according to their nationalities is shown in Table 1. The prevalence rate ranged from 0 to 21.4% with an average of 7.5%. Influenza A viruses were detected in pilgrims belonging to 12 different nationalities. The highest prevalence among recruited pilgrims was found in pilgrims from Turkey (21.4%), Indonesia (13.2%), Somalia (11.5%), and Nigeria (11.4%). In the latter groups, the prevalence rate was significantly higher than in those pilgrims coming from Australia, Britain, Ivory Coast, Sierra Leone, Syria, and the United States ($p < 0.05$).

Distribution of influenza-A-positive pilgrims according to their demographics

The distribution of the frequency of influenza-A-positive pilgrims in relation to demographic data is shown in Table 2. A significant association was found with age, the presence or absence of coughing, and smoking status. More specifically, there was a significantly higher prevalence rate of influenza A among young pilgrims (≤ 40 years of age) compared to older ones (> 40 years of age) ($p < 0.01$). In addition, pilgrims not showing any signs or symptoms of coughing had a significantly higher prevalence rate of infection with influenza A than those who did have coughing symptoms ($p < 0.05$). Moreover, smokers had significantly lower rates of infection with influenza A than did non-smokers ($p < 0.01$). There was no significant association between the rate of infection with the virus in relation to gender, flu vaccination status, presence or absence of sore throat, and antimicrobial usage at the time of sampling ($p > 0.05$).

2009 pandemic H1N1-positive pilgrims

H1N1pdm09 virus was detected in 9 of the 120 influenza-A-positive (7.5%) pilgrims. Of these nine pilgrims, two cases each were from Algeria, Indonesia, and Nigeria, and one each was from Britain, India, and Turkey. The distribution of the H1N1pdm09 virus was 50% higher in pilgrims below the age of 40 as compared to the older ones, in non-vaccinated pilgrims, in pilgrims showing no signs of sore throat or coughing, in pilgrims not using antimicrobials, and in non-smokers. Statistical analysis could not be performed on the data obtained from the H1N1pdm09-infected pilgrims and on the demographics of the low number of pilgrims who tested positive for the virus.

Discussion

The high prevalence of influenza A among pilgrims (7.5%) at the port of entry to KSA is alarming. The presence of influenza-A-positive individuals among pilgrims in the congregation of more than two million persons in a confined area raises the probability of transmitting to others and may result in the emergence of new, more virulent strains of the virus with more deleterious effects to public health [8,9]. The ethnic diversity of pilgrims, who came from around 165 countries, includes people with different protective immunities to the influenza A viruses with different subtypes and variants [30,31],

Table 1. Distribution of influenza A-positive pilgrims according to their nationalities listed in increasing order of statistical differences

Nationalities	No. of tested pilgrims	No. of influenza A- positive pilgrims (%)	No. of influenza A- negative pilgrims (%)
Australia	89	0 (0.0)[a]	89 (100.0)
Syria	110	0 (0.0)[a]	110 (100.0)
USA	99	0 (0.0)[a]	99 (100.0)
Britain	97	3 (3.0)[b]	94 (97.0)
Ivory Coast	128	3 (2.3)[b]	125 (97.7)
Sierra Leone	104	3 (2.9)[b]	101 (97.1)
Algeria	157	11 (7.0)[b,c]	146 (93.0)
India	188	12 (6.4)[b,c]	176 (93.6)
Somalia	113	13 (11.5)[c,d]	100 (88.5)
Nigeria	123	14 (11.4)[c,d]	109 (88.6)
Indonesia	280	37 (13.2)[d]	243 (87.8)
Turkey	112	24 (21.4)[e]	88 (78.6)
Total	1600	120 (7.5)	1,480 (92.5)

[a-e]Frequencies of influenza A-positive pilgrims in the column followed by different alphabetic superscripts are significantly different by Fisher's exact test ($p < 0.05$

resulting in varied susceptibilities of pilgrims to a wide spectrum of influenza strains. Actually, the data presented in Table 1 shows that certain pilgrims from different countries had no previous exposure to certain subtypes of influenza A viruses. This cluster of pilgrims who tested negative to influenza A viruses are most likely to be at risk of contracting such subtype [34]. The high prevalence of influenza A viruses encountered among Turkish, Indonesian, Nigerian, and Somali pilgrims at the port of entry to KSA compared to other nationalities could be because these pilgrims were exposed to the virus before their arrival at the airport. There was no significant correlation between the prevalence of influenza A in our study and the number of reported cases by WHO and other published studies [35-37].

The distribution of influenza-A-positive status among pilgrims, according to demographics (Table 2), showed significant association with three of the studied variables – presence of coughing, smoking, and age. Pilgrims with no cough had a significantly higher prevalence of influenza A compared to individuals with coughing symptoms ($p < 0.05$). This data is not in agreement with the known prominent clinical signs of coughing occurring in humans with influenza A infection [20]. A previous study found a positive correlation between the presence of coughing signs with the diagnosis of influenza A viruses [20]. Another significant difference was observed between smokers versus non-smokers; smokers had a lower prevalence rate of influenza A than did non-smokers ($p < 0.01$). To our knowledge, there is no report correlating smoking habits to the frequency of influenza infection. Most of the reported literature focused on the role of smoking on the enhancement of the deleterious effect of influenza-associated infections [25-29]. Given that most of our study group were smokers, the high prevalence rate of coughing among them could be chronic coughing due to their smoking habits. Future epidemiological studies should focus on comparing the frequency of influenza A in smokers versus non-smokers during targeted surveillances of influenza A viruses. This will shed more light on smoking habits in relation to influenza infections. The third significant correlation was noted between the presence of influenza A and age, with younger pilgrims (≤ 40 years of age) being most at risk ($p < 0.01$). Reports in the literature showed lower influenza A virus infections in older individuals, due to their wider acquired immunities to vaccines and/or

Table 2. Demographic distribution of influenza A-positive pilgrims according to significance of statistical differences

Significance of differences between the 2 categories under each of the 7 demographic variables	No. of tested pilgrims (N = 1,600)	No. of influenza A-negative pilgrims (%)	No. of influenza A-positive pilgrims (%)	P value*
Significant				
Coughing				
Present	1,483	1,379 (93.0)	104 (7.0)	P < 0.05
Absent	117	101 (86.3)	16 (13.7)	
Smoking				
Smoker	1,403	1,307 (93.2)	96 (6.8)	P < 0.01
Non-smoker	197	173 (87.8)	24 (12.2)	
Age (years)				
≤ 40	291	(85.6)	42 (14.4)	P < 0.01
> 40	1,309	1,231 (94.0)	78 (6.0)	
Not significant				
Antimicrobial usage				
Usage	176	158 (90.0)	18 (10.0)	P > 0.05
Non-usage	1,424	1,322 (92.8)	102 (7.2)	
Gender				
Female	603	554 (91.9)	49 (8.1)	P > 0.05
Male	997	926 (92.8)	71 (7.2)	
Vaccination				
Vaccinated	1,494	1,383 (92.6)	111 (7.4)	P > 0.05
Not vaccinated	106	97 (91.5)	9 (8.5)	
Sore Throat				
Present	61	55 (90.2)	6 (9.8)	P > 0.05
Absent	1,539	1,425 (92.6)	114 (7.4)	

*The P values reflect the significance of differences between the two categories under each demographic variable

field strains that they were exposed to during their lives [15,18].

No significant correlation was found between influenza A viruses and the remaining four variables in this study ($p > 0.05$), namely the use of antimicrobials, gender, flu vaccination, and the presence of sore throat. Antimicrobial usage against secondary bacterial infection seems to have no impact on influenza A infection. This is expected since influenza viruses are not susceptible to antibacterial drugs. The absence of significant correlation with gender differences is also expected since influenza A virus receptors are similar in both genders, with more inflammatory suppression in females due to the elevated 17B-estradiol [38].

The available documentations in the literature show differences between the two genders in their immune reactions during the onset of infection by influenza viruses [16-19]. Most literature showed that females had higher immune reactions to the virus than did males, with more significant immune injuries in pregnant women [17,19]. Vaccination against the flu among the surveyed pilgrims did not result in a significant drop in the prevalence of influenza A. Actually, most developing countries import flu vaccines from developed countries. The developing countries tailor their vaccines according to the mutated strains of influenza emerging at the end of the previous flu season [39]. These vaccines imported to developing countries might induce immunities that are not protective against their local non-characterized influenza A strains [40]. The prevalence of influenza A among pilgrims did not differ among individuals with sore throat versus those with the absence of this clinical sign ($p > 0.05$). This could be due to differences in the stage of the disease at the time of sample collection [41]. It is known that influenza A viruses could be present in the infected human for a certain number of days after the onset of symptoms [42-43].

Of the 120 pilgrims who tested positive for influenza A viruses, only 9 individuals (7.5%) were positive for the H1N1pdm09 virus. This prevalence was expected since the number of WHO reported cases of H1N1pdm09 dropped from 523,914 in 2009 to 54,168 in 2010, when this study was conducted [44]. The other 111 pilgrims were infected with other subtypes of the influenza A virus that the kits used did not have the specific primers to detect [45]. Future investigations should include screening for all subtypes and their mutated variants in pilgrims. The documentation of such data will be of global interest, due to the rich variety of ethnicities that represent the five continents of the globe. The 7.5% prevalence of H1N1pdm09 among our participating pilgrims could act as a reservoir for disseminating the infection to others in the mixed population at this huge congregation, considering that this H1N1pdm09 was able to circulate around the globe in only six weeks after emerging from Mexico in 2009 [9].

In agreement with what has been reported in the literature [15], six of the nine pilgrims who tested positive for H1N1pdm09 were ≤ 40 years of age. This is most likely due to differences in previous exposures to vaccines or field strains of cross-reacting viruses. The same number (6/9) of H1N1pdm09 was also observed in pilgrims who were not vaccinated against the flu. This is probably due to previous vaccination with protective H1N1 strains included in the vaccines marketed in the countries of the screened pilgrims. There is evidence of the efficiency of marketed vaccines in providing protection against the last circulating H1N1pdm09 virus [21]. Moreover, the prevalence of H1N1pdm09 in pilgrims with no clinical signs (coughing or sore throat) as compared to pilgrims manifesting the signs is most likely due to the time of oropharyngeal sampling, which could be at the end of the symptoms [42,43] or prior to the onset of symptoms as suggested by Roberts *et al*. [46], who concluded the possibility of H1N1pdm09 transmission in ferrets even before the appearance of symptoms and indicated that this conclusion should be considered for pandemic planning strategies.

The use of antibiotics did not seem to have an effect on the number of H1N1pdm09-positive pilgrims (8/9); this was expected since antibiotics are known to have no effect on this virus. Two out of the nine H1N1pdm09-positive pilgrims were smokers, although the number of positive H1N1pdm09-positive cases was too small to make a statistical conclusion; this result should, however, be considered in the background of the strong correlation of influenza-A-positive pilgrims and their non-smoking status, and needs to be studied further on a larger sample size. The main finding of the H1N1pdm09 data in this study is the potential of these influenza A viruses to be viable and be disseminated in enough quantities to be transmitted to other pilgrims in the highly crowded congregation with a high density that could reach up to eight pilgrims/m^2.

Conclusions

This study shows the presence of significant differences in the distribution of influenza-A-positive pilgrims based on their nationalities. The 7.5% of

pilgrims who tested positive for influenza A viruses from among the 1,600 screened pilgrims at the port of entry to KSA is indicative of the potential risk of these viruses being transmitted to others. There was a significant correlation between the prevalence of influenza A viruses in pilgrims in relation to age, absence of cough, and smoking habits. In addition, H1N1-positive pilgrims have the potential to transmit the infection to other pilgrims, which emphasizes the need for the development of a specific program that targets the prevention and control of influenza A viruses in this particular overcrowded gathering.

Future studies should include screening of the same persons in the incoming population during entry and just before their departure, after performing the Hajj. This will provide a better understanding of the transmission of influenza A subtypes among pilgrims and will help in establishing specific control measures to reduce the rate of transmission.

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Corresponding author

Esam Azhar
Department of Medical Laboratory Technology, Faculty of Applied Medical Science
King Abdulaziz University, Jeddah 21589, Saudi Arabia
Phone: +966566615222
Email: esam.azhar@yahoo.com

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