Circulation of Respiratory Viruses Among Pilgrims During the 2012 Hajj Pilgrimage

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Background. The Hajj is the oldest and largest annual mass gathering in the world and may increase the risk of spread of respiratory viruses.

Methods. We performed a prospective survey among a cohort of pilgrims departing from Marseille, France, to Mecca in the Kingdom of Saudi Arabia (KSA) for the 2012 Hajj season. Nasal swabs were collected from participants and tested for 11 respiratory viruses by real-time reverse transcription polymerase chain reaction.

Results. Of 165 participants sampled before departing to the KSA, 8 (4.8%) were positive for at least 1 virus (5 rhinovirus, 1 influenza C, 1 adenovirus, and 1 enterovirus). Seventy symptomatic pilgrims underwent additional nasal swabs during their pilgrimage in the KSA, of which 27 (38.6%) were positive for at least 1 virus (19 rhinovirus, 6 influenza A, 1 influenza C, 1 respiratory syncytial virus B, 1 metapneumovirus, 1 adenovirus, and 1 enterovirus). This was significantly higher than the 4.8% who were positive before departing for the KSA (P < .001). Of 154 pilgrims sampled before leaving the KSA, 17 (11%) were positive for at least 1 virus (13 rhinovirus, 3 adenovirus, 2 influenza B, and 1 enterovirus), which was also significantly higher than the percentage of positive pilgrims (4.8%), before departing for the KSA (P = .040).

Conclusions. This study suggests a rapid acquisition of respiratory viruses among pilgrims during their stay in the KSA, most notably rhinovirus, and highlights the potential of spreading these infections in the pilgrims’ home countries upon their return.

Keywords. cohort study; epidemiology; Hajj; respiratory tract infections; viruses.

Annually, nearly 2–3 million Muslims [1] from >180 countries across the globe gather in the Kingdom of Saudi Arabia (KSA) for a pilgrimage to the holy places of Islam known as the “Hajj.” Approximately 240 000 pilgrims arrive from the European region [2], including 30 000 pilgrims from France [3].

The Hajj and its rituals are physically demanding and usually last for 1 week, but the period of pilgrimage is up to 1 month. On arrival in Mecca, the holiest city in Islam, Hajj pilgrims start their pilgrimage by visiting the Grand Mosque for the circumambulation (Tawaf) of the Kaaba. The pilgrims then move, during subsequent days, to different sacred places located around the city of Mecca, including Mina, Arafat, and the Muzdalifah valleys, where they reside for several nights in tent camps [4] (Figure 1). Later, most pilgrims leave Mecca for the city of Medina to visit Islam’s second-holiest site, the Mosque of the Prophet.

The Hajj presents major public health and infection control challenges. In addition to fatigue and extreme weather conditions [4] (in Mecca, during October the
average temperature is >38°C during the day and >25°C at night, with a monthly rainfall averaging 3 mm), which increase the susceptibility of pilgrims to airborne infections, inevitable overcrowding within a confined area of individuals from different parts of the world and close contact with others greatly increase the risk of acquiring or spreading infectious diseases during the pilgrims’ stay [5]; in particular, acute respiratory infections, which are among the leading cause of acute illnesses worldwide, can spread among the pilgrims.

Respiratory tract infections are very common during the Hajj [1, 6] and account for most of the hospital admissions during this period [7, 8]. It has been estimated that more than one-third of pilgrims will experience respiratory symptoms during their stay [9]. A range of pathogens can cause acute respiratory infections, but respiratory viruses were found to be the most common etiology of upper respiratory tract infections among pilgrims in several surveys [1].

This study, including sample collection and laboratory methods, was conducted among a cohort of pilgrims departing from Marseille, France, to Mecca in the KSA for the 2012 Hajj season. They were tested for the most common respiratory viruses, with the aim of elucidating the dynamics of viral circulation among pilgrims.

**MATERIALS AND METHODS**

**Participants**
Participants were recruited between August and October 2012 from a private specialized travel agency in the city of Marseille, France, which organizes travel to Mecca. Pilgrims who planned to take part in the 2012 Hajj season were asked to participate in the study on a voluntary basis if they were 18 years of age or older and were able to provide consent.

**Study Design**
We conducted a cohort survey of participants who were followed up and sampled before departing to the KSA, during their pilgrimage in the KSA, and just before leaving the KSA. Upon inclusion, the participants were questioned by Arabic-speaking investigators using a standardized pretravel questionnaire, which included demographic data and medical history. A postravel questionnaire, which collected travel-associated diseases, vaccination status, and compliance with protective behaviors, was completed by a French Muslim Arabic-speaking medical doctor who traveled with the pilgrims. This survey was administered during a face-to-face interview just prior to returning to France or via telephone after returning to France. If we were unable to contact the pilgrim after 3 attempts, we considered the pilgrim lost to follow-up. Health problems that occurred during the stay in the KSA were monitored and recorded by the medical doctor. A cough was defined as the occurrence of a cough with or without sputum. Subjective fever was defined as a pilgrim’s report of feeling feverish. For the purpose of this study, influenza-like illness (ILI) was defined according to the presence of the triad of a cough, sore throat, and subjective fever [10]. This study was approved by our local ethics committee under number 13-017 and by the Saudi ethics committee. It was performed in accordance with the Good Clinical Practices recommended by the Declaration of Helsinki.
and its amendments. All participants gave written informed consent.

Respiratory Specimens
Anterior nare swabs were systematically collected from each participant using a commercial rigid cotton-tipped swab applicator (Sigma Virocult, MW950S, Wiltshire, England), in the month before departing from France and in the 3 days before leaving the KSA. In a number of cases, the medical doctor also collected additional nasal samples at the onset of symptoms during the pilgrimage in the KSA (within 2 weeks after their arrival). All the samples collected during the study were placed in viral transport media (Virocult virus transport medium) at the point of collection and kept at room temperature (stabilized by air conditioning to 20°C, in France and in the KSA) before being transported to the Marseille laboratory for storage in a −80°C freezer.

Detection of Respiratory Viruses
Each sample was tested for the following viruses by real-time reverse transcription polymerase chain reaction (rRT-PCR): influenza A (FLUA) [11], influenza B (FLUB) [11], influenza C (FLUC), and A/2009/H1N1 [12] viruses; human respiratory syncytial virus A and B (RSVB) [13]; human metapneumovirus (HMPV) [14]; human rhinovirus (HRV) [15]; human adenovirus (HAdV) [16]; and human enterovirus (HEV) [17].

Total nucleic acids were purified from a 400-μL sample volume and were spiked with MS2 + T4 bacteriophage as an internal control [18], using the BioRobot EZ1 XL with the Virus Mini kit v2.0 (both from Qiagen, Courtaboeuf, France) according to the manufacturer’s instructions. Each sample was tested independently in a 25-μL reaction containing 5 μL of RNA, 12.5 μL of 2× buffer (iScriptTM One-Step RT-PCR Kit for Probes, Bio-Rad), 1 μL of reverse transcriptase/Taq, 400 nM concentration of each primer, and 160 nM of probe. The reactions were performed using a C1000TM Thermal cycler (CFX96TM Real-Time System, Bio-Rad, Marnes-la-Coquette, France). The following cycling conditions were applied: 50°C for 10 minutes, followed by 95°C for 5 minutes; and then 45 cycles of 95°C for 15 seconds and 60°C for 30 seconds. The presence of inhibitors was determined using MS2 and T4 bacteriophage-specific detection systems, as previously reported [18].

Statistical Analysis
We hypothesized that several factors may influence the outcome of respiratory symptoms or virus portage during the stay, including age, preventive measures, and underlying chronic diseases. The Pearson χ² test and Fisher exact test, as appropriate, were applied to analyze the categorical variables. P values of ≤0.05 were considered significant. Statistical analyses were performed using SPSS software, version 17.2.

RESULTS

Characteristics of the Study Participants
A total of 169 participants were recruited to take part in the study, of whom 167 responded to the pretravel questionnaire. Table 1 summarizes their baseline demographics and characteristics. Participants’ mean age was 59.3 years (SD, 12.4; range, 21–83 years), with a male-to-female sex ratio of 0.6 to 1. They were predominately born in North Africa (90.4%), and most of the foreign-born individuals reported living in France for >20 years. Most of the participants reported living in Marseille and the surrounding cities. More than half of the participants (57.5%) reported suffering from at least 1 chronic disease, including diabetes (27.5%), hypertension (26.3%), chronic respiratory disease (7.8%), and chronic cardiac disease (7.2%).

Clinical Features
A total of 137 posttravel questionnaires were completed, representing a total response rate of 81.5%, and 46.0% of these were telephone-administered. The mean time between return from

| Table 1. Demographic and Baseline Characteristics of the Study Participants |
|---------------------------|---------|
| Characteristic            | No. (%) |
| Age group (n = 167)       |         |
| 20–40 y                   | 13 (7.8) |
| 41–60 y                   | 67 (40.1) |
| 61–80 y                   | 85 (50.9) |
| >80 y                     | 2 (1.2)  |
| Sex (n = 167)             |         |
| Male                      | 64 (38.3) |
| Female                    | 103 (61.7) |
| Birthplace (n = 167)      |         |
| Algeria                   | 116 (69.5) |
| Tunisia                   | 17 (10.2) |
| Morocco                   | 15 (9.0)  |
| Metropolitan France       | 13 (7.8)  |
| Other                     | 6 (3.6)   |
| Location of residence (n = 167) |         |
| Marseille                 | 110 (65.9) |
| Southern France (outside Marseille) | 48 (28.7) |
| Other                     | 7 (5.4)   |
| Duration of stay in France (n = 148) |       |
| 5–10 y                    | 8 (5.4)  |
| 10–20 y                   | 13 (8.8) |
| >20 y                     | 127 (85.8) |

* Among immigrants only.
the KSA and administration of the questionnaire by telephone was 24 days (range, 15–33 days).

Before departing to the KSA, none of the pilgrims presented acute respiratory symptoms at the time they were sampled. Pilgrims stayed in the KSA for 4 weeks, and the vast majority (90.4%) of them suffered from at least 1 respiratory symptom during their stay. A cough was the most frequently reported complaint (83.4% of respondents), followed by sore throat (79.7%), rhinorrhea (68.5%), myalgia (46.5%), feverishness (45.4%), dyspnea (19.6%), conjunctivitis (15.2%), and diarrhea (15.7%). Of the pilgrims who reported respiratory symptoms during their stay in the KSA, 41.3% met the criteria for self-reported ILI. The onset of respiratory symptoms peaked at 5–7 days after the arrival of the pilgrims in Mecca and declined thereafter. A second peak of smaller amplitude occurred on days 5 and 6 after arrival in Mina, shortly after moving from Arafat and Muzdalifah. Both peaks immediately occurred after performing the Tawaf in Mecca. Finally, only 2% of the pilgrims who were systematically sampled before leaving the KSA reported respiratory symptoms during the 3 days prior to leaving the KSA.

Regarding preventive measures, 45.6% of participants reported receiving a seasonal influenza vaccination in the past year and 35.9% reported receiving a pneumococcal vaccination in the past 5 years. During their stay in the KSA, 55.1% of pilgrims reported using face masks, 87.6% using disposable handkerchiefs, 40.3% frequent hand-washing, and 46.3% using hand sanitizer.

Pilgrims who reported frequent hand-washing during their stay, compared with those who reported using typical hand-washing habits, more frequently reported feverishness (55.6% vs 37.5%; odds ratio [OR], 2.08; 95% confidence interval [CI], 1.03–4.20; \( P = .039 \)) and ILI symptoms (53.7% vs 32.5%; OR, 2.41; 95% CI, 1.18–4.90; \( P = .014 \)). Rhinorrhea was more frequently reported by pilgrims who declared that they were not vaccinated against influenza in 2012 compared to vaccinated pilgrims (75.8% vs 56.8%; OR, 2.39; 95% CI, 1.14–5.01; \( P = .020 \)). Dyspnea was more frequently reported by those with chronic respiratory disease compared to other pilgrims (55.6% vs 17.3%; OR, 5.97; 95% CI, 1.48–24.02; \( P = .015 \)).

The majority of pilgrims (79.5%) sought healthcare from a doctor during their stay in the KSA, and 19.2% consulted a doctor after their return to France. Four pilgrims (3%) were hospitalized (1 in the KSA and 3 upon returning to France), 1 for respiratory tract infection, 1 for ILI symptoms, 1 for nephritic colic, and 1 for vomiting. No deaths occurred.

Overall Detection of Respiratory Viruses
Of the 169 participants enrolled in the study, 165 (97.6%) underwent a systematic pre-Hajj nasal swab before traveling to the KSA, and 154 (91.1%) underwent a systematic post-Hajj nasal swab in the 3 days before leaving the KSA (89.3% underwent both pre- and post-Hajj nasal swabs). A total of 70 pilgrims (41.4%) also underwent an additional nasal swab at the onset of acute respiratory symptoms during their pilgrimage in the KSA. The collection dates of the samples and results are shown in Figures 2, 3, and 4.

Before departing to the KSA, 8 participants (4.8%) were positive for at least 1 virus (5 HRV, 1 FLUC, 1 HAdV, and 1 HEV), without co-infection.

During the pilgrimage in the KSA, among the 70 symptomatic pilgrims who were sampled, 27 (38.6%) were positive for at least 1 virus (19 HRV, 6 FLUC, 1 RSVB, 1 HMPV, 1 HAdV, and 1 HEV), and there were 3 double infections (FLUA/FLUC, RSVB/HRV, and HRV/HEV). This was significantly higher than the 4.8% of pilgrims who were positive before departing to the KSA (\( P < .001 \)). The prevalence of HRV was significantly higher during the Hajj than before (27.1% vs 3%; \( P < .001 \)). Of the 19 pilgrims positive for HRV during their pilgrimage, 2 were positive before traveling to the KSA. Among the 57 pilgrims (41.0%) who met criteria for self-reported ILI during the Hajj, 40 (70.2%) were sampled, of whom 15 (37.5%) were virus-positive. The overall prevalence of respiratory viruses during the pilgrimage was significantly higher in pilgrims who reported more frequent hand-washing than usual during their stay compared to those who reported usual hand-washing (53.6% vs 23.3%; OR, 3.79; 95% CI, 1.23–11.69; \( P = .018 \)). No respiratory symptoms reported during the stay in the KSA were significantly associated with specific viral detection.

Before leaving the KSA, 17 pilgrims (11%) were positive for at least 1 virus (13 HRV, 3 HAdV, 2 FLUB, and 1 HEV), with 2 double infections (HRV/HAdV and HRV/HEV), and this was significantly higher than the 4.8% of pilgrims who were positive before departing to the KSA (\( P = .040 \)). The prevalence of HRV was significantly higher before leaving the KSA than before departing to the KSA (8.4% vs 3%; \( P = .036 \)). Of the 13 pilgrims positive for HRV before leaving the KSA, 3 were positive during their pilgrimage. The overall prevalence of respiratory viruses before leaving the KSA was significantly higher in individuals who reported using hand sanitizer during their stay compared to the remaining pilgrims (16.4% vs 5.6%; OR, 3.28; 95% CI, .97–11.07; \( P = .045 \)). All the pilgrims who were virus-positive before leaving the KSA complained of at least 1 respiratory symptom during their stay.

DISCUSSION
This is the first prospective longitudinal study investigating respiratory viruses from nasal specimens taken before departing for the KSA, during the pilgrimage in the KSA, and just before leaving the KSA in a single cohort of pilgrims, whether symptomatic or not. Other studies have been conducted either among...
symptomatic pilgrims recruited in the KSA [19, 20], among separate populations of arriving and departing pilgrims [21], and in returned pilgrims only [22], or were limited to the influenza virus [23–25].

Nine pilgrims out of 10 experienced respiratory symptoms during their stay in the KSA, with a cough and sore throat as the most common symptoms. ILI symptoms were reported by 41.0% of total pilgrims. An increase of respiratory symptoms was observed twice, following the Tawaf, which is performed in the Grand Mosque in Mecca in highly overcrowded conditions.

An 8-fold increase in the overall prevalence of respiratory viruses was observed between samples obtained before departing from France (4.8% of pilgrims) and samples obtained from ill pilgrims after performing their first Tawaf in Mecca (38.6% of pilgrims). In our study, HRV was the most frequent virus detected from symptomatic pilgrims (27%). This result is in accordance with studies conducted worldwide where HRV has been recognized as the most frequent virus responsible for the common cold in adults [26, 27]. This is not unexpected, as rhinoviruses are nonenveloped viruses that are more resistant in the environment than enveloped viruses. Symptoms of HRV infection are generally mild and limited to the upper respiratory tract. In contrast, lower respiratory symptoms associated with HRV infection are prominent in patients who have underlying asthma or other chronic lung disease [28]. In the present study, 7.8% of pilgrims reported chronic respiratory diseases; therefore, prevention must be reinforced in this high-risk population. There have been arguments [26] regarding whether rhinoviruses are spread chiefly from infected person to healthy individuals by direct contact to the fingers of healthy individuals by a handshake or indirect contact from the hands of an infected person to an intermediary surface [29], or through the aerosol route [30]. Our findings showed that more frequent hand-washing was significantly associated with feverishness and symptoms of ILI and with a higher prevalence of respiratory viruses. However, these results may indicate a reverse causation, as pilgrims with symptoms or who believe themselves to be at greater risk may have washed their hands more frequently. A recent study found that hand disinfection did not reduce HRV infection or HRV-related common cold illnesses [31]. The prevalence of HRV paralleled the increase of respiratory clinical symptoms, with a higher prevalence in pilgrims sampled in Mecca City during the first week of the pilgrimage. Influenza viruses ranked second after HRV among symptomatic pilgrims (10.0%), and 3 patients with influenza virus infection reported receiving a seasonal influenza vaccination prior to the Hajj pilgrimage.

Other viruses were rarely found in our study. We previously reported that the novel coronavirus Middle East respiratory
syndrome (MERS-CoV; formerly known as HCoV-EMC) was not detected during the 2012 Hajj season among the cohort pilgrims described here [32]. Other coronaviruses and parainfluenza viruses were not considered in this study and should be investigated in future studies.

More than 1 out of 10 pilgrims tested positive for overall respiratory viruses just before leaving the KSA, which indicates a high potential for spreading viral pathogens into pilgrims’ home countries. Given that a significant proportion of French pilgrims also travel to their country of birth in North Africa.

Figure 3. Patterns of reported respiratory symptoms and respiratory viruses detected during the stay in the Kingdom of Saudi Arabia (KSA). A. The respiratory symptoms reported by pilgrims during the stay in the KSA. B. Numbers of pilgrims sampled during the stay in the KSA with detection rate of total respiratory viruses. C. Daily numbers of cases of respiratory viruses detected during the stay in the KSA. Mean time of storage at 20°C was 22 days (range, 10–27 days) for samples collected in ill pilgrims during the pilgrimage in the KSA, and 6 days (range, 5–8 days) for post-Hajj samples collected just before leaving the KSA. 1: Arrival at Jeddah airport (afternoon) then Mecca (night). 2: Mecca. 3: Mecca then Mina (afternoon). 4: Arafat (morning) then Muzdalifah (night). 5: Muzdalifah then Mina (morning). 6: Mina, Mecca (morning) then Mina. 7: Mina. 8: Mina. 9: Medina (morning). 10: Departure from Jeddah airport. Abbreviations: FLUA, influenza A virus; FLUB, influenza B virus; FLUC, influenza C virus; HAdV, human adenovirus; HEV, human enterovirus; HMPV, human metapneumovirus; HRV, human rhinovirus; KSA, Kingdom of Saudi Arabia; RSVB, human respiratory syncytial virus B.
immediately after returning to France from the Hajj [33], there is also a potential for spreading these viruses beyond France.

The main limitation of this study is that, although the vast majority of pilgrims reported respiratory symptoms during their stay in KSA, the sampling by the medical doctor was limited to those who sought medical consultation from her. However, some symptomatic pilgrims were found to be negative for respiratory viruses. For those sampled at Mina and Medina, this was likely due to delayed medical consultation, as the specimen was collected several weeks after the onset of symptoms. Second, symptom occurrence was collected retrospectively and was based on self-reporting; thus, the date of the

Figure 4. The respiratory viruses detected among the Hajj pilgrims who tested positive during the study period. Abbreviations: FLUA, influenza A virus; FLUB, influenza B virus; FLUC, influenza C virus; H1N1, influenza A/2009/H1N1 virus; HAdV, human adenovirus; HEV, human enterovirus; HMPV, human metapneumovirus; HRV, human rhinovirus; KSA, Kingdom of Saudi Arabia; MS2, MS2 bacteriophage; RSVA, human respiratory syncytial virus A; RSVB, human respiratory syncytial virus B.
medical consultation did not necessarily correspond to the self-reported date of the onset of respiratory symptoms. Third, samples that were obtained at the beginning of the pilgrimage in the KSA were stored at room temperature (20°C) for periods up to 30 days before being processed, which may have been resulted in the degradation of genetic material. This may have likely contributed to underestimating the frequencies of infection. Alternative strategies to better preserve the samples must be considered, among which ethanol or nucleic acid lysis buffer [34] could be used. Fourth, a technical point is that HRV sequencing was not performed to determine how often new HRV infections were acquired during the stay in the KSA. Finally, we cannot demonstrate that the viruses detected from nasal swabs were responsible for the symptoms, as nasal carriage among pilgrims during their stay in the KSA, particularly rhinovirus, and demonstrates the potential for spreading these infections to pilgrims’ home countries upon their return.

Although our results cannot be extrapolated to all pilgrims, our study illustrates the rapid acquisition of respiratory viruses among pilgrims during their stay in the KSA, particularly rhinovirus, and demonstrates the potential for spreading these infections to pilgrims’ home countries upon their return.

Notes

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