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High prevalence of common respiratory viruses and no evidence of Middle East Respiratory Syndrome Coronavirus in Hajj pilgrims returning to Ghana, 2013

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Abstract

Objective: The Middle East respiratory syndrome coronavirus (MERS-CoV) emerged in 2012 on the Arabian Peninsula and has caused severe respiratory disease with more than 800 laboratory-confirmed cases. The return of infected pilgrims to their home countries with a putative spread of MERS-CoV necessitates further surveillance.

Methods: A surveillance study of 839 adult African Hajj pilgrims returning to Accra in Ghana, West Africa, was conducted in 2013 to assess the prevalence of respiratory symptoms as well as of MERS-CoV, human Rhinovirus (HRV), Respiratory Syncytial Virus (RSV), and Influenza A virus (FLU A) infection.

Results: 651 (77.6%) pilgrims had respiratory symptoms. Tests were positive for at least one of the viruses other than MERS-CoV in 179 (21.3%) of all pilgrims, with 22.4% detection in symptomatic vs. 17.6% detection in asymptomatic pilgrims. No MERS-CoV was detected, although common respiratory viruses were prevalent, with positive findings for HRV in 141 individuals (16.8%), RSV in 43 individuals (6.6%). This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/tmi.12482

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viduals (5.1%), and FLU A in 11 individuals (1.3%). Results were positive for more than one virus in 16 (1.9%) individuals, including 14 (1.7%) RSV/HRV co-infections and 2 (0.2%) FLU A/HRV co-infections. A total 146 (22.4%) of the symptomatic returnees tested positive for at least one respiratory virus compared with 33 (17.6%) of the asymptomatic pilgrims who had at least one detectable virus in their sample.

Conclusions: The prevalence of viral respiratory infections among Hajj pilgrims in both symptomatic and asymptomatic subjects is high. Although it is reassuring that MERS-CoV was not detected in the tested cohort, there is a need for active surveillance of Hajj pilgrims.

Keywords: MERS-Coronavirus, respiratory illness, Hajj pilgrimage, Human rhinovirus, Respiratory Syncytial virus, Influenza A virus

Introduction

Coronaviruses (CoV) in the genera *Alphacoronavirus* and *Betacoronavirus* [order *Nidovirales*, family *Coronaviridae*, subfamily *Coronavirinae*] infect a broad range of mammalian species including humans (Woo *et al.* 2009). While the human CoVs (HCoV)-HKU1, HCoV-229E, HCoV-NL63, and HCoV-OC43 cause mild to moderate respiratory tract infection, an epidemic of severe lower respiratory tract infections with a case fatality rate of approximately 10% was caused by SARS-CoV in 2002–2004 (Drosten *et al.* 2003). In 2012, a novel coronavirus termed Middle East Respiratory Syndrome-CoV (MERS-CoV) emerged on the Arabian Peninsula. MERS-CoV was found in more than 800 cases, with a steep increase case numbers in early 2014 (Zaki *et al.* 2012; de Groot *et al.* 2013; Drosten *et al.* 2014). Clinically, MERS-CoV presents mainly as a respiratory disease, with symptoms ranging from asymptomatic or mild upper respiratory tract disease to severe viral pneumonia and multi-organ failure. While most confirmed MERS-CoV cases originate from the Arabian Peninsula, several MERS-CoV infections have been imported to Europe (United Kingdom, Germany, Italy, France, Greece, Netherlands), Asia (Malaysia, Philippines), and the United States of America (Bermingham *et al.* 2012; Drosten *et al.* 2013; Mailles *et al.* 2013; Puzelli *et al.* 2013; Bialek *et al.* 2014; Kraaij-Dirkzwager *et al.* 2014; Premila Devi *et al.* 2014; Tsiodras *et al.* 2014).

All cases detected outside the Arabian Peninsula were linked to a Middle Eastern country either as a direct result of recent travel or through person-to-person transmission via a contact case. These imported cases highlight the fact that MERS-CoV, like other airborne viruses such as influenza, has the capability for worldwide spread.

Accepted Article

More than 10 million pilgrims from over 184 countries visit the Saudi Arabia (KSA) annually to perform religious pilgrimages to Mecca and Medina, either to perform the annual Hajj pilgrimage, which takes place during a certain season, or the Umrah (Memish *et al.* 2014). With the emergence of MERS-CoV on the Arabian Peninsula in 2012, this mass gathering is regarded as a possible transmission scenario with a risk of international spread of the virus (Khan *et al.* 2013).

There have been several surveillance studies for MERS-CoV in Hajj pilgrims, none of which has reported a MERS-CoV infection (Benkouiten *et al.* 2013; Gautret *et al.* 2013, Rashid *et al.* 2013, Gautret *et al.* 2014, Memish *et al.* 2014). No study on returning pilgrims from KSA to the African continent has been conducted, although the Muslim community in Africa consists of over 250 million people, with about one million annual travels to KSA for the pilgrimage (Zumla *et al.* 2014). We present our findings in screening returning pilgrims at Kotoka International airport in Accra, Ghana, one of West Africa's major intercontinental air travel hubs.

Materials and methods

Study design

We conducted a cross-sectional surveillance study in November 2013 at the Hajj Village located at the Kotoka International Airport (KIA), Ghana. The Hajj Village is a special arrival destination located on the premises of KIA for all chartered flights from KSA bringing Muslim pilgrims who embarked on the Hajj. Pilgrims were enrolled immediately after their return from the Hajj in KSA. Our goal was to identify the prevalence of MERS-CoV and the common respiratory viruses human Rhinovirus (HRV), Respiratory Syncytial Virus (RSV), and Influenza A virus (FLU A).

A standardised questionnaire on demographic and clinical data was completed during a face-to-face interview. Both symptomatic and asymptomatic subjects were recruited. Symptomatic subjects were defined as those presenting with any of the following conditions: cough, sore throat, breathing difficulty, runny nose, sneezing, or elevated temperature. All symptoms were self-reported. Asymptomatic subjects did not report any of the symptoms outlined above.

Sampling

Nasopharyngeal specimens were taken with flocked swabs (Copan, Italy) by inserting the swab up the nostril towards the pharynx until resistance was felt. Swabs were then rotated 3 times to obtain epithelial cells. The swabs were stored in 500 µl RNeasy lysis buffer (Qiagen, Hilden, Germany) and transported to the KCCR laboratory for extraction and testing by real-time reverse transcription polymerase chain reaction (real time RT-PCR).

Real-time RT-PCR analysis

RNA was purified using a Viral RNA Mini Kit (Qiagen) according to manufacturer instructions. All extracts were tested by real time RT-PCR using the Qiagen One-Step RT-PCR System (Qiagen) and assays with diagnostic sensitivity for HRV (Lu *et al.* 2008), RSV (Kuypers *et al.* 2004), FLU A (Spackman *et al.* 2002), and MERS-CoV (Corman *et al.* 2012, Corman *et al.* 2012). One-Step Real time (RT-) PCR detection was performed on a CFX96 Bio-Rad real-time PCR platform (Bio-Rad, Singapore). *In vitro* transcribed RNA was used as a positive control. In order to include only study subjects with a relevant viral load for HRV, RSV, and FLU A, only samples with a threshold cycle (CT)-value below 38 were rated as positive and included in our analysis.

Statistical analysis

Study data were double-entered into Excel and exported to Stata/SE 12 (Stata Corporation, Texas USA) for analysis. Pearson's χ^2 was used to analyse categorical variables where appropriate. P-values ≤ 0.05 were considered significant.

Ethics, consent, and approval

Approval for this study was obtained from the Public Health Division of the Ghana Health Services (GHS), Ministry of Health and the Port Health Directorate, Kotoka International Airport (KIA) also under the GHS. All participants were recruited on a voluntary basis. The aims and objectives of the study were explained to the pilgrims and verbal consent was obtained before participants were enrolled.

Results

Pilgrimage cohort

The cohort consisted of 839 adults recruited for the study in November 2013. The mean participant age was 52 years (range 21 to 85 years). The male-to-female ratio was 1:1.2 (Table). The pilgrims originated from all 10 geographic regions of Ghana with a majority from the Ashanti region (35.8%), followed by Greater Accra (22.5%), and the Northern Region (20.6%) (Figure 1).

Virus detection

Overall, 179 (21.3%) of the 839 screened individuals had a positive finding for at least one of the viruses other than MERS. HRV was the most common in 141 (16.8%) individuals, RSV in 43 (5.1%), and FLU A in 11 individuals (1.3%). In 16 (1.9%) pilgrims, more than one virus was detected with 14 (1.7%) RSV/HRV and 2 (0.2%) FLU A/HRV co-infections).

Clinical presentation

A total 651 (77.6%) of the pilgrims were symptomatic. Both, symptomatic and asymptomatic patients were of comparable age range and mean age (age range, 21 to 85 years; mean, 52 years and age range, 22 to 84 years; mean, 51 years, respectively).

The symptomatic returnees presented with cough (593, 91.1%), sore throat (343, 52.7%), elevated temperature (154, 23.7%), runny nose or sneezing (152, 23.3%), and breathing difficulty (124, 19.0%).

A total 146 (22.4%) of the symptomatic returnees tested positive for at least one respiratory virus compared with 33 (17.6%) of the asymptomatic pilgrims who had at least one detectable virus in their sample ($\chi^2 = 2.06$, $p < 0.001$).

Of the symptomatic pilgrims, 114 (17.5%) tested positive for HRV; 36 (5.5%) and 7 (1.1%) tested positive for RSV and FLU A, respectively. 27 (14.4%) of the asymptomatic returnees tested positive for HRV, 7 (3.7%) for RSV, and 4 (2.1%) were positive for FLU A. Differences between the individual virus and the two groups of pilgrims was not significant (Table).

Of the 593 pilgrims who presented with cough, 135 (22.8%) tested positive for at least one virus. Seventy-six (22.2%) of those who presented with sore throat also tested positive for at least one virus, while 33 (21.4%) of those who presented with elevated temperature also had a respiratory virus. Of those who presented with runny nose and breathing difficulty, 27 (17.8%) and 32 (25.8%) tested positive for at least one virus, respectively.

Discussion

Several surveillance studies on MERS-CoV in pilgrims returning from the annual Hajj have been performed after the emergence of MERS-CoV. In none of these studies, conducted during the last three Hajj seasons in 2012, 2013 and 2014, was MERS-CoV detected (Benkouiten *et al.* 2013, Gautret *et al.* 2013, Rashid *et al.* 2013, Gautret *et al.* 2014, Memish *et al.* 2014, Aberle *et al.*, 2014).

The first study after the emergence of MERS-CoV investigated 154 French Hajj pilgrims participating in the 2012 Hajj prior to returning to their home country for the presence of MERS-CoV in nasal swabs (Gautret *et al.* 2013). No MERS-CoV infection was detected but a high rate of respiratory symptoms was reported by 83.4% of pilgrims, with a subset of 41.0% fulfilling the criteria for influ-

enza-like illness (ILI), which is defined by cough, sore throat, and fever. However, this study did not test for viruses other than MERS-CoV. A second study from France assessed the rate of positive viral findings including MERS-CoV in a cohort of pilgrims before ($n = 165$), during ($n = 70$), and at the end (prior to the departure to their home country; $n = 154$) of their Hajj pilgrimage in the 2012 season (Benkouiten *et al.* 2013), without detection of MERS-CoV in any of the study subjects. The largest study on MERS-CoV prevalence in Hajj pilgrims so far was performed during the Hajj season of 2013 with a total of 5235 adult pilgrims from 22 countries, all sampled in KSA upon arrival (3210 pilgrims) or departure from the Hajj (2025). No MERS-CoV positive pilgrim was identified in this study; however, no other causes of viral illness have been assessed in this cohort (Memish, Assiri *et al.* 2014).

The role of respiratory infections during the Hajj is nevertheless of importance, independent of the emergence of MERS-CoV: Respiratory illness was the leading cause of hospital admissions at the Hajj (Al-Ghamdi *et al.* 2003). A review on available cross-sectional studies revealed an estimate between 20 to 80% of upper respiratory tract infection among Hajj pilgrims, with influenza and rhinoviruses as the most common viral agent (Al-Tawfiq *et al.* 2014). This is in line with findings from the above-mentioned studies on MERS-CoV in Hajj pilgrims: In the study by Benkouiten *et al.* (2013), the most commonly detected virus before, during, and at the end of the Hajj was HRV in 5/165 (3.0%), 19/70 (27.1%), and 13/154 (8.4%), respectively. A significantly higher number of viral infections during and at the end of the Hajj were seen with 27/70 (38.6%) individuals positive for at least one respiratory virus during the Hajj and 17/154 (11.0%) pilgrims positive at the end of the Hajj compared to 8/165 (4.8%) pilgrims who were positive before their departure from France to KSA. (Benkouiten *et al.* 2013). This suggests a probable rapid acquisition of respiratory viruses among pilgrims during their stay. Benkouiten *et al.* (2013) report the detection of FLU A during the Hajj in 6/70 (8.6%) pilgrims, but did not detect FLU A at the end of the Hajj. In contrast, we found 1.3% of pilgrims positive for FLU A upon return to Ghana. In contrast to our data, Benkouiten reported only a few RSV infections with a positive finding for RSV in only 1/70 (1.4%) pilgrim during the Hajj. Despite the slightly different sampling time point (upon departure in KSA and after return to Ghana), further reasons for the differences between studies may be the small number reported in the cohorts, with only 165, 70, and 154 pilgrims in the French study (Benkouiten *et al.* 2013) versus our cohort of 839 individuals. The different ethnic and geographic background of the cohort, and therefore a different pre-existing immunity may also account for the observed differences. There may also have been a difference between the seasons (2012 Hajj season versus 2013 Hajj season). Notably, in a follow-up study performed during the 2013 Hajj season, the same group reported a higher prevalence of influenza than previously found, with 10/129 pilgrims testing positive for influenza viral RNA (8 influenza

A (H3N2), 1 for influenza A (H1N1), 1 for influenza B). Furthermore, a high rate of respiratory symptoms was found with 117/129 (90.7%) of pilgrims reporting respiratory symptoms while still in KSA and 55/129 (78.6%) in the 3–5 weeks after their return (Gautret *et al.* 2014), but no other respiratory viruses were screened for in the study.

Interestingly, a study assessing the aetiology of severe community-acquired pneumonia in returning Hajj pilgrims showed 21/26 (80.7%) pilgrims in whom a respiratory pathogen (viruses or bacteria) was detected were positive for a viral finding (Memish *et al.* 2014). Similar to our cohort, the most common respiratory virus was HRV, detected in 57.7% of positive samples, followed by FLU A in 23.1%. MERS-CoV was not the cause of severe CAP in any of the hospitalised pilgrims investigated.

Our data support the findings from earlier studies on lack of MERS-CoV infections in returning pilgrims during the 2013 Hajj season. However, in light of the rapidly increasing number of MERS-CoV cases in early 2014 (more than 800 documented cases thus far) and the upcoming 2015 Hajj season, further surveillance is necessary to confirm the absence of MERS-CoV transmission in the 2015 pilgrimage cohort. This is of utmost importance considering the severe overcrowding situations during these mass gatherings. In developing countries with limited resources for molecular diagnostics and a diverse spectrum of febrile aetiologies, early detection of MERS-CoV infected patients will remain a challenge.

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Figure legend

Figure 1. Regional map of Ghana showing the geographical distribution of the Hajj pilgrims.

Table. Characteristics of the study population.

Characteristic	Symptomatic, n (%)	Asymptomatic, n (%)	χ^2 (p-value)
Number	651 (77.6)	188 (22.4)	
Mean age, range	52, 21-85	51, 22-84	
Age groups			10.22 (0.116)
21-30	27 (4.3)	12 (6.5)	
31-40	112 (17.9)	40 (21.6)	
41-50	131 (21.0)	41 (22.2)	
51-60	187 (29.9)	40 (21.6)	
61-70	133 (21.3)	37 (20.0)	
>71	35 (5.6)	15 (8.1)	
Missing	26 (4.0)	3 (1.6)	
Sex			0.74 (0.389)
Male	292 (44.9)	91 (48.4)	
Female	359 (55.2)	97 (51.6)	
Region of residence			8.59 (0.476)
Ashanti	226 (34.7)	74 (39.4)	
Brong Ahafo	25 (3.8)	7 (3.7)	
Central Region	19 (2.9)	3 (1.6)	
Eastern Region	16 (2.5)	1 (0.5)	
Greater Accra	149 (22.9)	40 (21.3)	
Northern Region	131 (20.1)	42 (22.3)	
Upper East Region	20 (3.0)	5 (2.7)	
Upper West Region	32 (4.9)	12 (6.4)	
Volta	17 (2.6)	2 (1.0)	
Western Region	16 (2.5)	2 (1.0)	
Virus detection			
HRV	114 (17.5)	27 (14.4)	1.035 (0.31)
RSV	36 (5.5)	7 (3.7)	0.979 (0.32)
Flu A	7 (1.1)	4 (2.1)	1.249 (0.26)

