

Lack of nasal carriage of novel corona virus (HCoV-EMC) in French Hajj pilgrims returning from the Hajj 2012, despite a high rate of respiratory symptoms

P. Gautret^{1,2}, R. Charrel^{1,3}, K. Belhouchat¹, T. Drali¹, S. Benkouiten¹, A. Nougairede^{1,3}, C. Zandotti^{1,3}, Z. A. Memish^{4,5}, M. al Masri⁴, C. Gaillard¹, P. Brouqui^{1,2} and P. Parola^{1,2}

1) IHU Méditerranée Infection, APHM Public Hospitals of Marseille, 2) Unité de Recherche en Maladies Infectieuses et Tropicales Emergentes (URMITE), Faculté de Médecine, WHO Collaborative Centre for Rickettsioses and Other Arthropod Borne Bacterial Diseases, Aix Marseille Université, UM63, CNRS 7278, IRD 198, Inserm I095, 3) EHESP French School of Public Health, Aix Marseille Université, IRD French Institute of Research for Development, Marseille, France, 4) WHO Collaborating Centre for Mass Gathering Medicine, Public Health Directorate, Saudi Ministry of Health and 5) College of Medicine, Alfaisal University, Riyadh, Saudi Arabia

Abstract

A cohort of 154 French Hajj pilgrims participating in the 2012 Hajj were systematically sampled with nasal swabs prior to returning to France, and screened for the novel HCoV-EMC coronavirus by two real-time RT-PCR assays. Despite a high rate of respiratory symptoms (83.4%), including 41.0% influenza-like illness, no case of HCoV-EMC infection was detected. Despite the fact that zoonotic transmission was suspected in the first few cases, a recent family cluster in the Kingdom of Saudi Arabia suggests that the virus might show at least limited spread from person to person, which justifies continuing epidemiological surveillance.

Keywords: Coronavirus, Hajj pilgrimage, HCoV-EMC, screening

Original Submission: 16 December 2012; **Revised**

Submission: 23 January 2013; **Accepted:** 23 January 2013

Editor: T. A. Zupanc

Article published online: 11 February 2013

Clin Microbiol Infect 2013; **19**: E315–E317

10.1111/1469-0691.12174

Corresponding author: P. Gautret, IHU Méditerranée Infection, APHM Public Hospitals of Marseille, 13005 Marseille, France
E-mail: philippe.gautret@club-internet.fr

To date, nine human cases of severe respiratory infections caused by a previously unknown coronavirus called HCoV-EMC (for Erasmus Medical Centre) have been documented

since June 2012 [1–5]. Five cases, three of which were fatal, occurred in Saudi Arabian citizens. Two cases occurred in Qatari citizens, including one who travelled to Saudi Arabia 2 weeks before the onset of the disease. Two fatal cases occurred in Jordan. So far, only the three most recently confirmed cases in Saudi Arabia have been epidemiologically linked—they are from the same family, living in the same household. No secondary cases were observed in the hospital among doctors and nurses caring for the patients, which suggests that the disease did not spread readily. A history of animal contact with camels, sheep and bats has been documented.

These nine cases of HCoV-EMC infection may represent occasional transmission of the virus from animals to humans, manifesting as severe infections with no subsequent human-to-human transmission. Despite the apparently low transmissibility of HCoV-EMC at this stage, it may also be the beginning of another SARS-like pandemic, in which there will be increasing animal-to-human and subsequent human-to-human transmission [6]. Alternatively, these nine documented cases may just represent the tip of an iceberg, with a majority of cases remaining undetected because of mild or asymptomatic forms of infection [6]. From a public health point of view, serious attention should be paid to the surveillance of influenza-like illnesses in the Middle East region, as the extremely crowded Hajj (pilgrimage to Makkah) season has just ended [7].

This article reports a prospective cohort study carried in Saudi Arabia in October–November 2012. Participants in the survey were Hajj pilgrims travelling with one specialized travel agency in Marseille. Pilgrims older than 18 years were included on a voluntary basis, and participants were asked to sign a written consent form. Follow-up was conducted by a French Muslim Arabic-speaking medical doctor during the journey period, who systematically documented travel-associated diseases (medical consultation, hospitalization, fever, cough, and other respiratory symptoms). Nasal swabs were obtained from all of the study participants: (i) just before leaving France to travel to Saudi Arabia; and (ii) just before returning from Saudi Arabia to France, regardless of their medical condition. Additional samples were obtained during the stay in Saudi Arabia from symptomatic patients, in a number of cases. Samples were placed in viral transport medium at the point of collection, kept at room temperature, and transported to the Marseille laboratory for storage at -80°C . Total nucleic acids were purified from a 200- μL volume of sample spiked with MS2 + T4 bacteriophage as internal control [8], by use of the BIOROBOT EZI XL with the Virus Mini kit v2.0 (both from Qiagen, Courtaboeuf, France). Each sample was tested independently with the two systems developed by Corman *et al.* [9] in a 25- μL reaction containing 5 μL of RNA, 12.5 μL of 2X

buffer (iScript™ One-Step RT-PCR Kit for Probes (BioRad, Marnes-la-Coquette, France)), 1 µL of reverse transcriptase/Taq, 400 nM each primer, and 160 nM probe. The cycling profile (50°C for 10 min, followed by 95°C for 5 min, and then 45 cycles of 95°C for 15 s and 60°C for 30 s) was obtained on BioRad CFX 96 cyclers. The limit of detection was tested at 90 and 820 genome equivalents per reaction for the ORF1b and the UpE system, respectively. Ethical approval was obtained from the Saudi authorities.

A total of 169 pilgrims were included on departure from France. The mean age was 59.3 years (range: 21–83 years), and the male/female ratio was 0.6. Most of them were born in North Africa (90.4%) and had settled in France >20 years before (85.8%). A proportion of 57.5% declared suffering from a chronic disease, including diabetes (27.5%), hypertension (26.3%), chronic respiratory infection (7.8%), and chronic cardiac disease (7.2%). A proportion of 45.6% declared having received influenza vaccination in 2012, before participating in the Hajj. Clinical data were available for 157 individuals (92.9%), of whom 90.4% suffered from respiratory symptoms during their stay in Saudi Arabia, including cough (83.4%) and sore throat (79.7%); 41.0% met the criteria for influenza-like illness (ILI) as defined by the association of cough, sore throat, and fever. One patient only was hospitalized (ILI + diarrhoea). A total of 70 (41.4%) pilgrims who suffered from respiratory symptoms had a nasal swab taken while they had symptoms. One hundred and fifty-four pilgrims (91.1%) had a systematic nasal swab taken during the 3 days before returning to France. All PCRs were negative for HCoV-EMC, with the two RT-PCR assays. These patients were tested with the two initially reported RT-PCR systems (ORF1b and UpE).

The detection limit of our adapted tests is different from that reported by Corman *et al.* [9], probably because of technical differences (equipment and RT-PCR kit). However, the sensitivity of the combined tests is satisfactory.

The purpose of our work was not to investigate patients meeting the case definition for suspicion of HCoV-EMC infection [10], but to investigate the potential circulation of HCoV-EMC in Hajj pilgrims independently of the occurrence of respiratory symptoms. Clinical data from the 2012 cohort show higher rates of cough (83.4% vs. 48.5%), sore throat (79.9% vs. 36.1%) and ILI (41.0% vs. 8.0%) than in a previous study conducted on French pilgrims during the 2009 Hajj [11]. This may be attributable to better follow-up of pilgrims in 2012, which was probably greatly helped by the presence of a medical doctor together with pilgrims during the Hajj; the 2009 survey was conducted by telephone interview after pilgrims had returned to France. This may also account for an actual higher rate of respiratory infections in 2012. Seasonal influenza vaccine is recommended for Hajj pilgrims by the

French Ministry of Health [12]. The lower influenza vaccination rate among pilgrims of the 2012 cohort, recruited at a travel agency (40.3%), than among pilgrims of the 2009 cohort, recruited when they were receiving pre-travel advice at our clinic (97.4%) [11], may also possibly account for the higher rate of ILI in 2012 than in 2009. Our results are based on relatively small numbers of individuals, and are therefore not representative of all pilgrims participating in the Hajj. However, the absence of HCoV-EMC among French pilgrims despite a very high attack rate of cough episodes suggests that the virus did not play a major role in the epidemiology of Hajj-associated respiratory infections during 2012.

At present, the very few available data on the behaviour of HCoV-EMC do not support human-to-human transmission of the virus, and suggest that zoonotic transmission is likely. However, the recent family cluster in the Kingdom of Saudi Arabia suggests that the virus might show at least limited spread from person to person, which justifies continuing epidemiological surveillance. Further investigations are being conducted to identify other pathogens among the 2012 French pilgrim cohort.

Transparency Declaration

The authors state they have no conflict of interest.

References

1. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N Engl J Med* 2012; 367: 1814–1820.
2. Bermingham A, Chand M, Brown C *et al.* Severe respiratory illness caused by a novel coronavirus, in a patient transferred to the United Kingdom from the Middle East, September 2012. *Euro Surveill* 2012; 17, pii: 20290.
3. Pebody R, Chand M, Thomas H *et al.* The United Kingdom public health response to an imported laboratory confirmed case of a novel coronavirus in September 2012. *Euro Surveill* 2012; 17, pii: 20292.
4. Albarrak AM, Stephens GM, Hewson R, Memish ZA. Recovery from severe novel coronavirus infection. *Saudi Med J* 2012; 33: 1265–1269.
5. World Health Organization. *Novel coronavirus infection—update 30 November 2012*. http://www.who.int/csr/don/2012_11_30/en/index.html
6. Chan JF, Li KS, To KK, Cheng VC, Chen H, Yuen KY. Is the discovery of the novel human betacoronavirus 2c EMC/2012 (HCoV-EMC) the beginning of another SARS-like pandemic? *J Infect* 2012; 65: 477–489.
7. Al-Ahdal MN, Al-Qahtani AA, Rubino S. Coronavirus respiratory illness in Saudi Arabia. *J Infect Dev Ctries* 2012; 6: 692–694.
8. Ninove L, Nougairède A, Gazin C *et al.* RNA and DNA bacteriophages as molecular diagnosis controls in clinical virology: a comprehensive study of more than 45 000 routine PCR tests. *PLoS ONE* 2011; 6: e16142.

9. Corman VM, Eckerle I, Bleicker T *et al.* Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. *Euro Surveill* 2012; 17, pii: 20285.
10. Al-Tawfiq J, Memish Z. The Hajj: updated health hazards and current recommendations for 2012. *Euro Surveill* 2012; 17, pii: 20295.
11. Gautret P, Vu HV, Sani S, Douchi M, Parola P, Brouqui P. Protective measures against acute respiratory symptoms in French pilgrims participating in the Hajj of 2009. *J Travel Med* 2011; 18: 53–55.
12. Haut Conseil de la Santé Publique. Health recommendations for travellers, 2012. *Bull Epid Hebdo* 2012; 20–21: 225–253.