Acquisition of respiratory viruses and presence of respiratory symptoms in French pilgrims during the 2016 Hajj: A prospective cohort study

Van-Thuan Hoang, Doudou Sow, Fabiola Dogue, Sophie Edouard, Tassadit Drali, Saber Yezli, Badriah Alotaibi, Didier Raoult, Philippe Parola, Vincent Pommier de Santi, et al.

Acquisition of respiratory viruses and presence of respiratory symptoms in French pilgrims during the 2016 Hajj: a prospective cohort study

Van-Thuan Hoang[1,2*], Doudou Sow[1,3*], Fabiola Dogue[1], Sophie Edouard[4], Tassadit Drali[1], Saber Yezli[5], Badriah Alotaibi[5], Didier Raoult[4], Philippe Parola[1], Vincent Pommier de Santi[1,6], Philippe Gautret[1]

[1]Aix Marseille Univ, IRD, AP-HM, SSA, VITROME, IHU-Méditerranée Infection, Marseille, France

[2]Thai Binh University of Medicine and Pharmacie, Viet Nam

[3]Service de Parasitologie-Mycologie, Faculté de médecine, Unversité Cheikh Anta Diop, Dakar, Senegal

[4]Aix Marseille Univ, IRD, APHM, MEPHI, IHU-Méditerranée Infection, Marseille, France

[5]The Global Centre for Mass Gatherings Medicine, Ministry of Health, Riyadh, Saudi Arabia

[6]French Military Center for Epidemiology and Public Health Marseille, France

*Equal work

Corresponding author:

Philippe Gautret

VITROME, Institut Hospitalo-Universitaire Méditerranée Infection, 19-21 Boulevard Jean Moulin 13385 Marseille Cedex 05, France. Phone: + 33 (0) 4 13 73 24 01. Fax: + 33 (0) 4 13 73 24 02. E-mail address: philippe.gautret@club-internet.fr

Abstract

Background: Viral respiratory tract infections are frequent among Hajj pilgrims. However, it is still not known whether viruses are responsible for the symptoms observed in sick pilgrims or whether they only colonize sick and asymptomatic pilgrims.

Patients and methods: A prospective cohort study was conducted among French Hajj pilgrims in 2016. Medical follow-up and systematic nasal swabbing were performed pre- and post-Hajj. Additional samples were obtained per-Hajj, at symptom onset in ill pilgrims. Viruses were identified using the BioFire FilmArray® Respiratory multiplex qualitative PCR panel.

Results: 109 pilgrims were included. 83.5% presented respiratory symptoms during Hajj and 39.5% were still symptomatic on return. 5.5% of pre-Hajj, 95.2% of per-Hajj (at symptom onset) and 46.5% of post-Hajj samples tested positive ($p<0.0001$). Acquisition rates of rhinovirus/enterovirus, coronavirus 229E and influenza A virus were respectively 38.6%, 19.8% and 2.0%. Although rhinovirus/enterovirus, coronavirus 229E and influenza A clearance were respectively 70.6%, 71.4% and 100% on return, overall virus carriage proportion on return was 75.0% in pilgrims with influenza-like illness and 44.0% in those who have never experienced this symptoms or resolved it (OR = 4.05, 95% CI [1.02 - 16.02]).

Conclusions: Viruses likely play some role in the pathogenesis of the respiratory tract infections at the Hajj. Point of care-rapid multiplex PCR assays are valuable diagnosis tools in this context when used at respiratory symptom onset or soon after.

Keywords

Hajj; mass gathering; respiratory tract infection; viruses; PCR

Introduction

Hajj pilgrimage has long been associated with enhanced transmission of infectious disease agents. Epidemics of cholera [1] and bacterial meningitis [2,3] are emblematic examples of the potential for international spread of life-threatening infections at the Hajj, given its international component with pilgrims originating from up to 180 countries and gathering in Mecca before returning to their home country [4]. More recently, respiratory tract infections at the Hajj have attracted the attention of the medical community because of the frequency of respiratory symptoms among pilgrims consulting at primary health care facilities or hospitalized in Saudi Arabia [5]. Cohort studies conducted in populations of pilgrims originating from different countries reported that a substantial proportion of pilgrims suffer from respiratory symptoms during their stay in Saudi Arabia [6]. On the other hand, numerous PCR-based studies have demonstrated the frequent acquisition of respiratory viruses following participation in Hajj [7,8]. Fortunately, SARS-CoV and MERS-Cov did not affect Hajj pilgrims so far [9,10]. However, rhinovirus, non-MERS coronaviruses and influenza viruses are commonly isolated from both asymptomatic returning pilgrims and pilgrims with acute respiratory symptoms [7,8]. Due to the lack of detailed clinical information in many Hajj studies and the high sensitivity of PCR tools, the contribution of viruses to observed symptoms remains unknown.

The objective of the study was to evaluate the nasal carriage of respiratory viruses before and after traveling to the Hajj, and to investigate a possible relationship between viral carriage and respiratory symptoms with a careful clinical follow-up in a cohort of French Hajj pilgrims departing from Marseille.

Materials and methods

Study population

The Figure 1 details the procedure of this study. The study was conducted among French Hajj pilgrims travelling together to Mecca, from August 27th to September 20th, 2016, 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. Upon inclusion, the participants were questioned using a standardized pre-travel questionnaire that included demographic and chronic disease data and vaccination status. Health issues were recorded by a medical doctor who travelled with the group of pilgrims. We considered that participants suffered RTIs if they presented with cough and/or rhinitis and/or sore throat. Influenza like illness (ILI) was defined as the association of cough, sore throat and fever [11]. Each individual was classified in one of the three categories: i. asymptomatic (those who did not experience any respiratory symptoms during the entire stay in Saudi Arabia), ii. resolved respiratory tract infection (RTI) (those who experienced respiratory symptoms including cough and/or sore throat and/or rhinitis and/or voice failure during travel, but who recovered at the time of return to France and iii. ongoing RTI (those with ongoing respiratory symptoms at the time of leaving Saudi Arabia).

The protocol was approved by our Institutional Review Board (July 23, 2013; reference No. 2013-A00961-44). It was performed in accordance with the good clinical practices recommended by the Declaration of Helsinki and its amendments. All participants gave a written informed consent.

Sample collection

The procedure included a systematic nasal swab 10 days before departing from France (pre-Hajj specimens) and just 1 day (on September 19th, 2016) before leaving the KSA (Kingdom

of Saudi Arabia) (post-Hajj specimens) We previously showed that nasal swabs are more sensitive than pharyngeal swabs in detecting respiratory viruses in Hajj pilgrims, using real-time reverse transcriptase-polymerase chain reaction methods [12]. Nasal swabs were also performed among symptomatic pilgrims who spontaneously consulted the accompanying medical doctor at the time of onset (per-Hajj specimens). No per-Hajj sample was collected among asymptomatic pilgrims. Samples were collected using commercial rigid cotton-tipped swab applicators (Medical Wire & Equipment, Wiltshire, UK) which were inserted in the anterior nose and then placed in viral transport media (Sigma Virocult®). This standardized procedure was previously explained to the pilgrims by the investigators. The swabs were stored at 20°C before being transported to the Marseille laboratory for storage in a freezer at -80°C within 48 hours of collection for pre- and post-Hajj samples. Per-Hajj specimen were kept at 20°C until the return to France.

Identification of respiratory pathogens

The analyses were carried out in Marseille, following return (thus results were not available during the stay in Saudi Arabia) with a validated multiplex qualitative PCR method [13]. The BioFire respiratory panel (BFRP, BioFire) includes the following virus targets: rhinovirus/enterovirus, adenovirus, human coronavirus (229E, HKU1, NL63 and OC43), human metapneumovirus, influenza virus A and B, parainfluenza virus (1, 2, 3 and 4) and respiratory syncytial virus. Three bacteria are targeted in the test including *Bordetella pertussis, Chlamydia pneumoniae and Mycoplasma pneumoniae.* Acquisition of pathogens was defined as the absence of a given pathogen in pre-Hajj samples and the presence of this pathogen in per- and or post-Hajj samples.

Statistical analysis

The Pearson's Chi-square test and Fisher's exact test, as appropriate, were applied to analyze the categorical variables. To evaluate the potential acquisition of respiratory viruses in Saudi Arabia, we used the McNemar's Test to compare their percentage before leaving France and in Saudi Arabia. Percentages and odds ratio (OR) with 95% confidence interval (CI) estimations and comparisons were carried out using the STATA 11.1 (Copyright 2009 StataCorp LP, http://www.stata.com). P values of .05 or less were considered significant.

Results

Demographics, and respiratory symptoms

A total of 109 pilgrims were included on departure from France. The median age was 63 years (interquartile: 55-69, range: 23–96), and 46.8% were males. 11 (10.1%) individuals reported a history of chronic respiratory disease and only one pilgrim had respiratory symptoms before leaving Marseille. At total, 26/109 (23.8%) pilgrims received influenza vaccination during the past 12 months, but only 2/109 (1.8%) reported having been vaccinated against pneumococcal (PCV-13) in the last 5 years. A total of 91 (83.5%) pilgrims presented at least one respiratory symptom during their stay in Saudi Arabia with cough, rhinitis and sore throat being most frequent (Table 1). The median time between arrival to Saudi Arabia and symptom onset was 9 days (range: 0-18 days). The majority of pilgrims had their onset of symptoms during their stay in Mecca and Mina (Figure 2). Twenty-five (22.9%) pilgrims had influenza like illness. 30 (27.5%) pilgrims took antibiotics for the purpose of respiratory tract infection symptoms, based on the clinical judgment of the accompanying doctor and none was hospitalized. Forty-three (39.5%) were still symptomatic at the time of leaving Saudi Arabia.

Acquisition of virus and bacteria carriage

Nasal swabs were obtained from 109 pilgrims (100%) before traveling to Saudi Arabia, from 21 patients at symptom onset in Saudi Arabia and from 99 (90.8%) pilgrims before leaving Saudi Arabia for France. Among these, 99 individuals (90.8%) underwent paired samples allowing the calculation of acquisition rates. The proportion of pilgrims positive for each potential pathogen detected is presented in Table 2. Of the pre-Hajj samples, 6 (5.5%) were positive, while a pathogen was found in 20 (95.2%) per-Hajj samples and in 46 (46.5%) post-Hajj samples ($p<0.0001$). Overall, the acquisition rate of at least one pathogen during the stay in Saudi Arabia was 40.0%. The proportion of pilgrims positive for rhinovirus/enterovirus and coronavirus 229E were significantly higher in post-Hajj samples, compared to pre-Hajj samples, with acquisition rates of respectively 38.6% and 19.8%. Multiple infections were frequent accounting for 50.0% positive per-Hajj samples and 17.4% of positive post-Hajj samples with rhinovirus/enterovirus-coronavirus 229E mixed infection the most common (Table 3). No case was positive for the three bacteria tested.

Virus carriage according to respiratory symptoms

Among 108 asymptomatic pilgrims sampled in France before travel, 5/108 (4.6%) were positive for rhinovirus/enterovirus. The only pilgrim suffering respiratory symptoms before travel was positive for rhinovirus/enterovirus.

Among the 21 pilgrims sampled at symptom onset in Saudi Arabia, 20 tested positive for at least one virus with high proportion of rhinovirus/enterovirus (81.0%), coronavirus 229E (33.0%) and influenza A (23.8%) (Table 2). Seven of the 21 symptomatic pilgrims had ILI and their carriage rate did not significantly differ from that of other ill pilgrims (data not shown). Of the 21 pilgrims sampled at the time of symptom onset, 19 were resampled before leaving Saudi Arabia, allowing the proportion of individuals who allowed their viral transport back to the country to be calculated. The mean time between onset of symptoms and testing

on leaving KSA was 15.4 days (ranging 8-19). Rhinovirus/enterovirus carriage was cleared in 70.6.1% cases, coronavirus 229E carriage in 71.4% cases and influenza virus A carriage in 100% cases, while the majority of cases were still symptomatic (71.4%) (Figure 3).

Comparison of virus carriage at the time of return, according to the presence of respiratory symptoms at sampling time showed a highest proportion of virus positivity (at least one virus) in symptomatic patients (57.9%) compared to those who have never experienced cough or who resolved it (39.3%), but the difference was not significant (OR = 2.11, 95% CI[0.93 - 4.83] (Tables 4). However, carriage of at least one virus on return was significantly higher in patients with ongoing ILI symptoms (75.0%) compared to patients without ILI symptoms or with resolved ILI symptoms (42.5%), OR = 4.05, 95%CI [1.02 - 16.02] (Table 5). Breakdown by virus type; however, did not show statistically significant variations, although their percentage of positivity was higher in pilgrims presenting with at least one respiratory symptom or ILI symptoms at the time of sampling, in comparison with those who did not. Comparison of virus carriage in patients who experienced symptoms during the Hajj (ongoing and resolved) and in patients who were asymptomatic at any time did not show significant differences.

Discussion

We observed both a high frequency of respiratory symptoms during the pilgrims' stay in KSA (76.2% cough and 22.9% ILI) and a significant acquisition of viral nasal carriage (40.0%), mostly due to rhinovirus/enterovirus and coronavirus 229E. This corroborates the results obtained from most recent studies conducted among French pilgrims by our team and among pilgrims from other nationalities [14-22]. The epidemic curves of onset of symptoms and virus carriage in samples obtained at onset of symptoms suggest an early acquisition of respiratory virus during the initial stay in Mecca, probably due to inter-human transmission as

attested by the bi-modal pattern of the curves during this period of time. Crowded conditions at Al-Haram Mosque during the rituals, with up to 8 individuals per m^2 recorded close to the Kaaba [23], is likely to play a significant role in this process. Asymptomatic carriage of rhinovirus/enterovirus and coronaviruses was frequently observed when tested in returning pilgrims. Nevertheless, overall viral carriage in patients with ILI on return (75.0%) was significantly higher than in individuals without ILI (42.5%), which suggests that viruses play a role in the pathogenesis of the RTIs. Viruses were detected in almost all 21 pilgrims sampled at symptom onset, which reinforces this view. However, while most of these 21 pilgrims were still symptomatic at the post-Hajj sampling time; only a low proportion of those testing positive at onset remained positive in post-Hajj samples two weeks after first symptoms. These results, although based on small numbers of ill pilgrims clearly demonstrate that sampling at the time of leaving KSA results in underestimation of viral carriage in relation with symptoms during the stay because the majority of ill pilgrims already cleared their viral infection despite persisting symptoms, as observed in our survey. Since obtaining respiratory samples at onset of symptoms is challenging in the context of longitudinal cohort survey at the Hajj, most studies conducted so far were based on systematic samples obtained at the time of return, days after the onset of symptoms occurred. Such a design evaluating viral carriage at return provides useful on the potential for respiratory viruses to spread upon return to the country of origin, assuming that some of the viruses detected by PCR are viable. It is however less appropriate for retrospectively investigating the responsibility of viruses in respiratory symptoms experienced during the stay in Saudi Arabia, given the rapid virus clearance. This was particularly obvious in our survey with a proportion of influenza A virus positivity of 2.0% in pilgrims screened on return, regardless of symptoms contrasting with 20% in pilgrims who actively consulted our medical investigator sampled at respiratory symptom onset. Studies conducted in different countries from 2009 through 2015 with the

aim of screening influenza virus by PCR among returning pilgrims regardless of symptoms, reported a mean influenza carriage rate of 3.4%, ranging 0.4-7.8% [14,15,19,23-26], which is in line with the results reported here (1.9%). When similar screening studies were conducted, enrolling only pilgrims with respiratory symptoms on returning to their home country, a higher proportion of 10.6% influenza carriage was observed, ranging 3.0-14.5% when we found a slightly lower proportion of 2.1% in this study [27-31]. Finally, in studies conducted in sick pilgrims seen in Saudi or European hospitals for suspected MERS-Cov infection and therefore suffering from more severe respiratory symptoms, the percentage of confirmed influenza infection was 34.9%, ranging from 12.0 to 71.4% [21,32-35].

Our study has some limitations. First, it was conducted among a small number of pilgrims during one season of Hajj, and our results cannot be extrapolated to all pilgrims. Secondly, we were able to collect respiratory samples at symptom onset in only one fourth of patients who reported suffering respiratory symptoms during the study period. This low proportion is due to the nature of the recruitment since sampling at onset of symptoms was not systematic but performed in patients who spontaneously consulted the accompanying doctor at the early beginning of their illness. The sample type used is not FDA cleared or CE marked and the BFRP, BioFire performance characteristics using nasal swabs instead of nasopharyngeal swabs have not been established.

Conclusion

Viruses are acquired by the vast majority of Hajj pilgrims soon after their arrival in Mecca and likely responsible for respiratory symptoms, notably ILI. Viral clearance is rapid. Point of care-rapid multiplex PCR assays are valuable diagnosis tools for Hajj patients when used at respiratory symptom onset or soon after. In particular, they allow the detection of the

influenza virus, which is particularly interesting because it has practical consequences for the early prescription of antivirals in people at risk. These tests are also useful for ruling-out MERC-CoV infection and deciding about isolation measure lifting.

Acknowledgement

This work was supported by bioMérieux and by the Agence Nationale de la Recherche (programme "investissement d'avenir" Méditerranée Infection 10-IAHU-03), the Région Provence-Alpes-Côte d'Azur and European funding FEDER PRIMI.

References

1. Ahmed QA, Arabi YM, Memish ZA. Health risks at the Hajj. Lancet. 2006;367:1008-15. https://doi.org/ 10.1016/S0140-6736(06)68429-8
2. Yezli S, Assiri AM, Alhakeem RF, Turkistani AM, Alotaibi B. Meningococcal disease during the Hajj and Umrah mass gatherings. Int J Infect Dis. 2016;47:60-4. https://doi.org/ 10.1016/j.ijid.2016.04.007
3. Yezli S. The threat of meningococcal disease during the Hajj and Umrah mass gatherings: A comprehensive review. Travel Med Infect Dis. 2018;24:51-8. https:// doi.org/10.1016/j.tmaid.2018.05.003.
4. Memish ZA, Zumla A, Alhakeem RF, Assiri A, Turkestani A, Al Harby KD, et al. Hajj: infectious disease surveillance and control. Lancet. 2014;383:2073-82. https://doi.org/ 10.1016/S0140-6736(14)60381-0.
5. Al-Tawfiq JA, Zumla A, Memish ZA. Respiratory tract infections during the annual Hajj: potential risks and mitigation strategies. Curr Opin Pulm Med. 2013;19:192-7. https://doi.org/ 10.1097/MCP.0b013e32835f1ae8.

6. Al-Tawfiq JA, Gautret P, Benkouiten S, Memish ZA. Mass gatherings and the spread of respiratory infections. Lessons from the Hajj. Ann Am Thorac Soc. 2016;13:759–65. https://doi.org/ 10.1513/AnnalsATS.201511-772FR.
7. Gautret P, Benkouiten S, Al-Tawfiq JA, Memish ZA. Hajj-associated viral respiratory infections: A systematic review. Travel Med Infect Dis. 2016;14:92-109. https://doi.org/ 10.1016/j.tmaid.2015.12.008.
8. Al-Tawfiq JA, Benkouiten S, Memish ZA. A systematic review of emerging respiratory viruses at the Hajj and possible coinfection with Streptococcus pneumoniae. Travel Med Infect Dis. 2018;23:6-13. https://doi.org/ 10.1016/j.tmaid.2018.04.007.
9. Al-Tawfiq JA, Zumla A, Memish ZA. Travel implications of emerging coronaviruses: SARS and MERS-CoV. Travel Med Infect Dis. 2014;12:422-8. https://doi.org/ 10.1016/j.tmaid.2014.06.007.
10. Gautret P, Benkouiten S, Al-Tawfiq JA, Memish ZA. The spectrum of respiratory pathogens among returning Hajj pilgrims: myths and reality. Int J Infect Dis. 2016;47:83-5. https://doi.org/ 10.1016/j.ijid.2016.01.013.
11. Rashid H, Shafi S, El Bashir H, Haworth E, Memish ZA, Ali KA, et al. Influenza and the Hajj: defining influenza-like illness clinically. Int J Infect Dis. 2008;12:102-3. https://doi.org/ 10.1016/j.ijid.2007.03.009.
12. Benkouiten S, Gautret P, Belhouchat K, Drali T, Nougairede A, Salez N, et al. Comparison of nasal swabs with throat swabs for the detection of respiratory viruses by real-time reverse transcriptase PCR in adult Hajj pilgrims. J Infect. 2015;70:207-10. https://doi.org/10.1016/j.jinf.2014.08.011.
13. Leber AL, Everhart K, Daly JA, Hopper A, Harrington A, Schreckenberger P, et al. Multicenter Evaluation of BioFire FilmArray Respiratory Panel 2 for Detection of

Viruses and Bacteria in Nasopharyngeal Swab Samples. J Clin Microbiol. 2018;56. pii: e01945-17. https://doi.org/ 10.1128/JCM.01945-17.

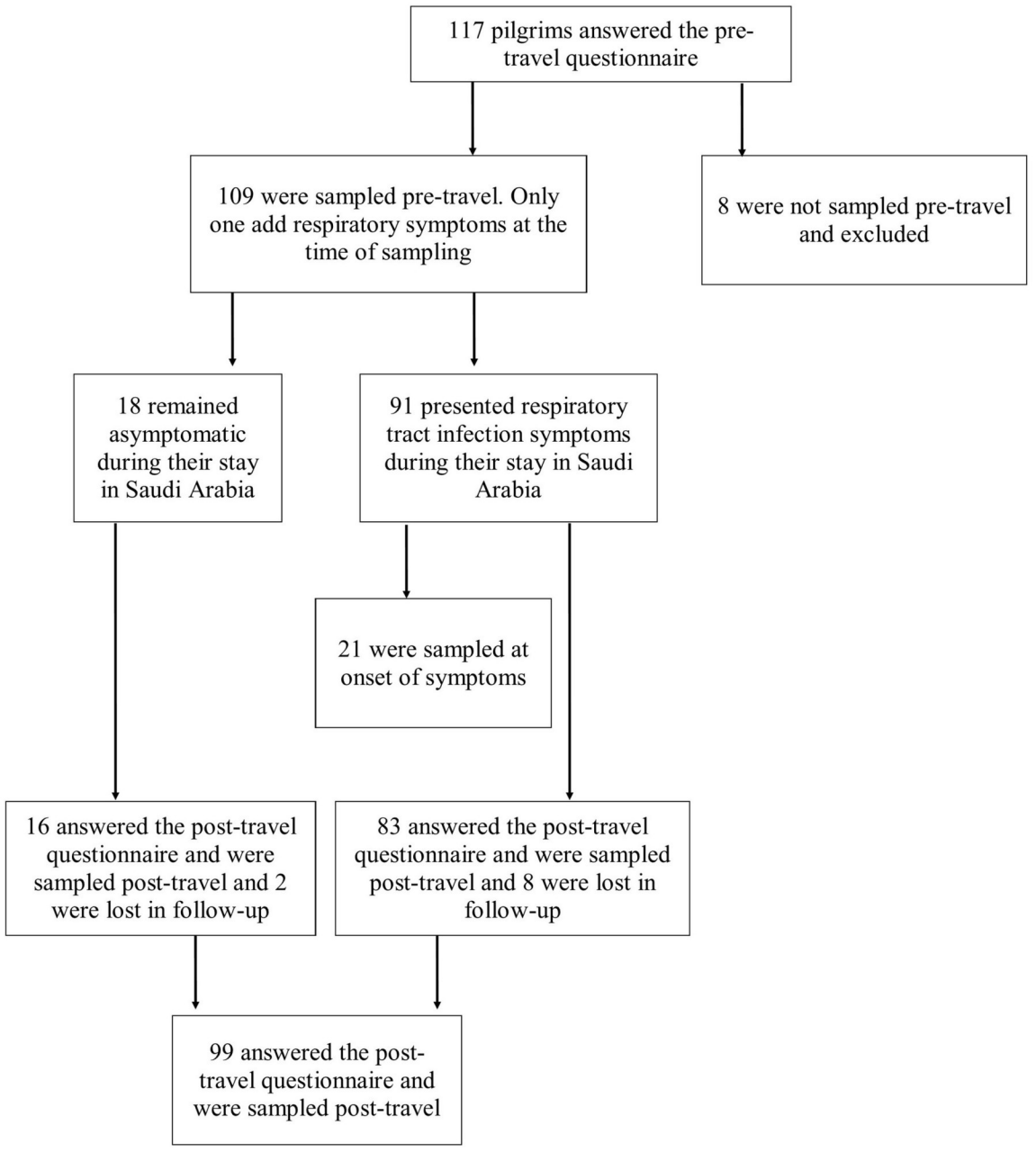
14. Benkouiten S, Charrel R, Belhouchat K, Drali T, Salez N, Nougairede A, et al. Circulation of respiratory viruses among pilgrims during the 2012 Hajj pilgrimage. Clin Infect Dis. 2013;57:992-1000. https://doi.org/10.1093/cid/cit446.
15. Benkouiten S, Charrel R, Belhouchat K, Drali T, Nougairede A, Salez N, et al. Respiratory viruses and bacteria among pilgrims during the 2013 Hajj. Emerg Infect Dis. 2014;20:1821-7. https://doi.org/ 10.3201/eid2011.140600.
16. Rashid H, Shafi S, Haworth E, El Bashir H, Memish ZA, Sudhanva M, et al. Viral respiratory infections at the Hajj: comparison between UK and Saudi pilgrims. Clin Microbiol Infect. 2008;14:569-74. https://doi.org/ 10.1111/j.1469-0691.2008.01987.
17. Memish ZA, Assiri AM, Hussain R, Alomar I, Stephens G. Detection of respiratory viruses among pilgrims in Saudi Arabia during the time of a declared influenza A(H1N1) pandemic. J Travel Med. 2012;19:15-21. https://doi.org/ 10.1111/j.1708-8305.2011.00575.x.
18. Barasheed O, Rashid H, Alfelali M, Tashani M, Azeem M, Bokhary H, et al. Viral respiratory infections among Hajj pilgrims in 2013. Virol Sin. 2014;29:364-71. https://doi.org/ 10.1007/s12250-014-3507-x.
19. Memish ZA, Assiri A, Turkestani A, Yezli S, Al Masri M, Charrel R, et al. Mass gathering and globalization of respiratory pathogens during the 2013 Hajj. Clin Microbiol Infect. 2015;21:571.e1-8. https://doi.org/10.1016/j.cmi.2015.02.008.
20. Annan A, Owusu M, Marfo KS, Larbi R, Sarpong FN, Adu-Sarkodie Y, et al. High prevalence of common respiratory viruses and no evidence of Middle East respiratory syndrome coronavirus in Hajj pilgrims returning to Ghana, 2013. Trop Med Int Health. 2015;20:807-12. https://doi.org/10.1111/tmi.12482.

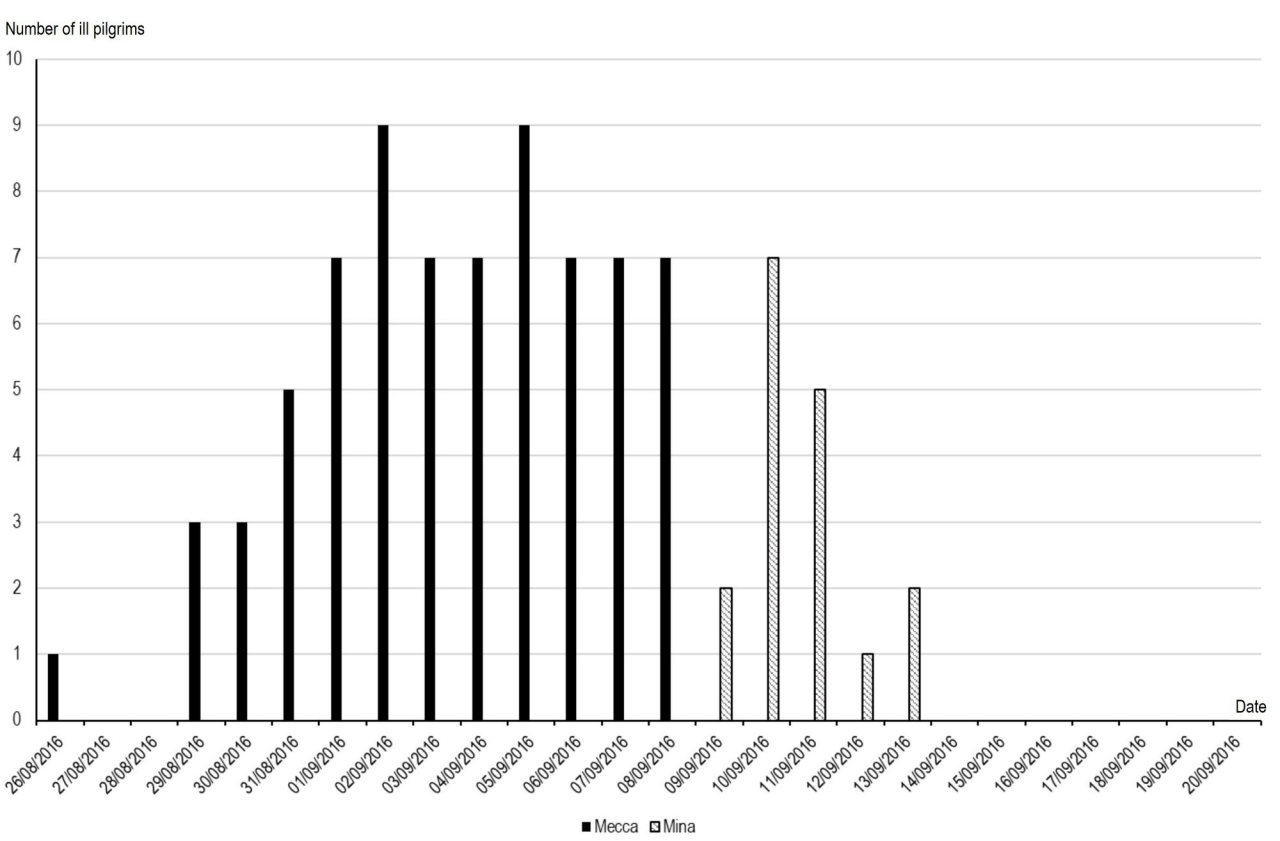
21. Atabani SF, Wilson S, Overton-Lewis C, Workman J, Kidd IM, Petersen E, et al. Active screening and surveillance in the United Kingdom for Middle East respiratory syndrome coronavirus in returning travellers and pilgrims from the Middle East: a prospective descriptive study for the period 2013-2015. Int J Infect Dis. 2016;47:10-4. https://doi.org/10.1016/j.ijid.2016.04.016.
22. Alnabulsi H, Drury J. Social identification moderates the effect of crowd density on safety at the Hajj. Proc Natl Acad Sci U S A. 2014;111:9091-6. https://doi.org/10.1073/pnas.1404953111.
23. Kandeel A, Deming M, Elkreem EA, El-Refay S, Afifi S, Abukela M, et al. Pandemic (H1N1) 2009 and Hajj Pilgrims who received Predeparture Vaccination, Egypt. Emerg Infect Dis. 2011;17:1266-8. https://doi.org/10.3201/eid1707.101484.
24. Memish ZA, Assiri AM, Hussain R, Alomar I, Stephens G. Detection of respiratory viruses among pilgrims in Saudi Arabia during the time of a declared influenza A(H1N1) pandemic. J Travel Med. 2012;19:15-21. https://doi.org/10.1111/j.1708-8305.2011.00575.x.
25. Ziyaeyan M, Alborzi A, Jamalidoust M, Moeini M, Pouladfar GR, Pourabbas B, et al. Pandemic 2009 influenza A (H1N1) infection among 2009 Hajj Pilgrims from Southern Iran: a real-time RT-PCR-based study. Influenza Other Respir Viruses. 2012;6:e80-4. https://doi.org/10.1111/j.1750-2659.2012.00381.x.
26. Ma X, Liu F, Liu L, Zhang L, Lu M, Abudukadeer A, et al. No MERS-CoV but positive influenza viruses in returning Hajj pilgrims, China, 2013-2015. BMC Infect Dis. 2017;17:715. https://doi.org/10.1186/s12879-017-2791-0.
27. Moattari A, Emami A, Moghadami M, Honarvar B. Influenza viral infections among the Iranian Hajj pilgrims returning to Shiraz, Fars province, Iran. Influenza Other Respir Viruses. 2012;6:e77-9. https://doi.org/10.1111/j.1750-2659.2012.00380.x.

28. Refaey S, Amin MM, Roguski K, Azziz-Baumgartner E, Uyeki TM, Labib M, et al. Cross-sectional survey and surveillance for influenza viruses and MERS-CoV among Egyptian pilgrims returning from Hajj during 2012-2015. Influenza Other Respir Viruses. 2017;11:57-60. https://doi.org/ 10.1111/irv.12429.
29. Koul PA, Mir H, Saha S, Chadha MS, Potdar V, Widdowson MA, et al. Influenza not MERS CoV among returning Hajj and Umrah pilgrims with respiratory illness, Kashmir, north India, 2014-15. Travel Med Infect Dis. 2017;15:45-47. https://doi.org/10.1016/j.tmaid.2016.12.002.
30. Al-Abdallat MM, Rha B, Alqasrawi S, Payne DC, Iblan, Binder AM, et al. Acute respiratory infections among returning Hajj pilgrims-Jordan, 2014. J Clin Virol. 2017;89:34-37. https://doi.org/10.1016/j.jcv.2017.01.010.
31. Muraduzzaman AKM, Khan MH, Parveen R, Sultana S, Alam AN, Akram A, et al. Event based surveillance of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in Bangladesh among pilgrims and travelers from the Middle East: An update for the period 2013-2016. PLoS One. 2018;13:e0189914. https://doi.org/ 10.1371/journal.pone.0189914.
32. Aberle JH, Popow-Kraupp T, Kreidl P, Laferl H, Heinz FX, Aberle SW. Influenza A and B Viruses but Not MERS-CoV in Hajj Pilgrims, Austria, 2014. Emerg Infect Dis. 2015;21:726-7. https://doi.org/10.3201/eid2104.141745.
33. Griffiths K, Charrel R, Lagier JC, Nougairede A, Simon F, Parola P, et al. Infections in symptomatic travelers returning from the Arabian peninsula to France: A retrospective cross-sectional study. Travel Med Infect Dis. 2016;14:414-6. https://doi.org/10.1016/j.tmaid.2016.05.002.
34. Yavarian J, Shafiei Jandaghi NZ, Naseri M, Hemmati P, Dadras M, Gouya MM, et al. Influenza virus but not MERS coronavirus circulation in Iran, 2013-2016: Comparison

between pilgrims and general population. Travel Med Infect Dis. 2018;21:51-5. https://doi.org/10.1016/j.tmaid.2017.10.007.

35. Assiri AM, Asiri SI, Banassir T, Baljoon MJ, Jokhdar H. Burden of influenza-related severe acute respiratory infections during Hajj season 1438 (2017). Lessons and future directions. Saudi Med J. 2018;39:524-5.


117 pilgrims answered the pre-travel questionnaire
109 were sampled pre-travel. Only one add respiratory symptoms at the time of sampling
8 were not sampled pre-travel and excluded
18 remained asymptomatic during their stay in Saudi Arabia
91 presented respiratory tract infection symptoms during their stay in Saudi Arabia
21 were sampled at onset of symptoms
16 answered the post-travel questionnaire and were sampled post-travel and 2 were lost in follow-up
83 answered the post-travel questionnaire and were sampled post-travel and 8 were lost in follow-up
99 answered the post-travel questionnaire and were sampled post-travel


Number of ill pilgrims
10
9
8
7
6
5
4
3
2
1
0
Date
26/08/2016
27/08/2016
28/08/2016
29/08/2016
30/08/2016
31/08/2016
01/09/2016
02/09/2016
03/09/2016
04/09/2016
05/09/2016
06/09/2016
07/09/2016
08/09/2016
09/09/2016
10/09/2016
11/09/2016
12/09/2016
13/09/2016
14/09/2016
15/09/2016
16/09/2016
17/09/2016
18/09/2016
19/09/2016
20/09/2016
Mecca
Mina

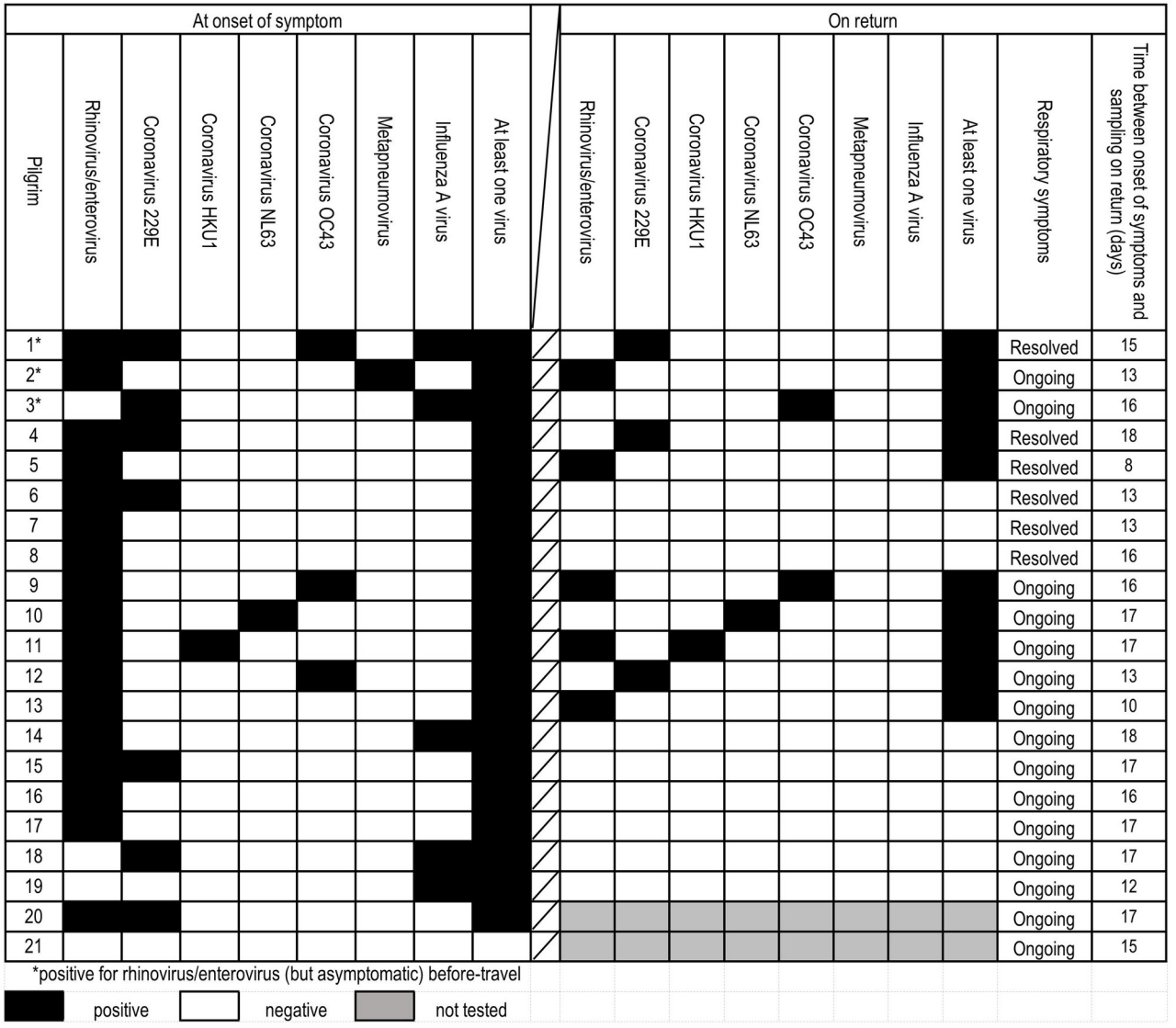

At onset of symptom
On return
Pilgrim
Rhinovirus/enterovirus
Coronavirus 229E
Coronavirus HKU1
Coronavirus NL63
Coronavirus OC43
Metapneumovirus
Influenza A virus
At least one virus
Respiratory symptoms
Time between onset of symptoms and sampling on return (days)
1* Resolved 15
2* Ongoing 13
3* Ongoing 16
4 Resolved 18
5 Resolved 8
6 Resolved 13
7 Resolved 13
8 Resolved 16
9 Ongoing 16
10 Ongoing 17
11 Ongoing 17
12 Ongoing 13
13 Ongoing 10
14 Ongoing 18
15 Ongoing 17
16 Ongoing 16
17 Ongoing 17
18 Ongoing 17
19 Ongoing 12
20 Ongoing 17
21 Ongoing 15
*positive for rhinovirus/enterovirus (but asymptomatic) before-travel
positive
negative
not tested

Table 1. Clinical symptoms in 109 participants

Symptoms	n (%)
At least one respiratory symptom	91 (83.5)
Cough	83 (76.2)
Expectoration	39 (35.8)
Dry cough	44 (40.4)
Rhinitis	62 (56.9)
Sore throat	61 (56.0)
Voice failure	39 (35.8)
Dyspnea	26 (23.9)
Fever	28 (25.7)
Myalgia	40 (36.7)
Conjunctivitis	3 (2.8)
Influenza like illness	25 (22.9)
Time between arrival in Kingdom of Saudi Arabia and onset of respiratory symptoms	
Median	9
Interquartile	7 - 12
Range	0 - 18

Table 2. Frequency of pathogens detected in pre-, per- and post-Hajj samples

	Pre-Hajj (N = 109) Number of individuals with positive sample (percentage)	Per-Hajj (N = 21) Number of individuals with positive sample (percentage)	Post-Hajj (N = 99) Number of individuals with positive sample (percentage)	Acquisition rate (pre- versus post-Hajj) (N = 99)	p value (pre- versus post-Hajj)*
Rhinovirus/enterovirus	6 (5.5)	17 (81.0)	32 (32.3)	28 (38.6)	<0.0001
Adenovirus	0	0	3 (3.0)	3 (3.0)	0.08
Coronavirus 229E	0	7 (33.3)	15 (15.2)	15 (19.8)	<0.0001
Coronavirus HKU1	0	1 (4.8)	1 (1.0)	1 (1.0)	0.32
Coronavirus NL63	0	1 (4.8)	1 (1.0)	1 (1.0)	0.32
Coronavirus OC43	0	3 (14.3)	2 (2.0)	2 (2.0)	0.15
Metapneumovirus	0	1 (4.8)	0	0 (0)	-
Influenza A	0	5 (23.8)	2 (2.0)	2 (2.0)	0.15
Influenza B	0	0	0	0	-
Parainfluenza virus type 1	0	0	0	0	-
Parainfluenza virus type 2	0	0	0	0	-
Parainfluenza virus type 3	0	0	0	0	-
Parainfluenza virus type 4	0	0	0	0	-

Respiratory syncitial virus	0	0	0	0	-
At least one virus	6 (5.5)	20 (95.2)	46 (46.5)	40 (40.0)	<0.0001

*McNemar's Test

Table 3. Frequency of carriage of multiple pathogen combinations

	Pre-Hajj n (%) N = 6	Per-Hajj n (%) N = 20	Post-Hajj n (%) N = 46
Rhinovirus/enterovirus + adenovirus	-	-	3 (6.5)
Rhinovirus/enterovirus + coronavirus 229E	-	5 (25.0)	4 (8.7)
Rhinovirus/enterovirus + coronavirus HKU1	-	1 (5.0)	1 (2.2)
Rhinovirus/enterovirus + coronavirus NL63	-	1 (5.0)	-
Rhinovirus/enterovirus + coronavirus OC43	-	3 (6.0)	1 (2.2)
Rhinovirus/enterovirus + metapneumovirus	-	1 (2.0)	-
Rhinovirus/enterovirus + influenza A	-	2 (4.0)	1 (2.2)
Coronavirus 229E + Influenza A	-	3 (6.0)	-
Rhinovirus/enterovirus + adenovirus + coronavirus 229E	-	-	1 (2.2)
Rhinovirus/enterovirus + adenovirus + coronavirus OC43	-	-	1 (2.2)
Rhinovirus/enterovirus + coronavirus 229E + OC43	-	1 (2.0)	-
Rhinovirus/enterovirus + coronavirus 229E + OC43 + Influenza A	-	1 (2.0)	-
Total samples with multiple pathogens detected	-	10 (50.0)	8 (17.4)

Table 4. Frequency of pathogens in post-Hajj samples according to respiratory symptoms at the time of sampling.

		Asymptomatic (N = 16)	Resolved symptoms* (N = 45)	Ongoing symptoms* (N = 38)	OR [95%CI], P value (ongoing symptoms versus asymptomatic or resolved symptoms)	OR [95%CI], P value (ongoing or resolved symptoms versus asymptomatic)
Rhinovirus/enterovirus	Yes	5 (31.3)	13 (28.9)	14 (36.8)	1.39 [0.59 - 3.29], P = 0.45	1.06 [0.34 – 3.36], P = 0.92
	No	11 (68.7)	32 (71.1)	24 (63.2)		
Adenovirus	Yes	0 (0)	1 (2.2)	2 (5.3)	3.33 [0.30 - 38.08], P = 0.33	-
	No	16 (100)	44 (97.8)	36 (94.7)		
Coronavirus 229E	Yes	0 (0)	8 (17.8)	7 (18.4)	1.50 [0.49 - 4.53], P = 0.48	-
	No	16 (100)	37 (82.2)	31 (81.6)		
Coronavirus HKU1	Yes	0 (0)	0 (0)	1 (2.6)	-	-
	No	16 (100)	45 (100)	37 (97.4)		
Coronavirus NL63	Yes	0 (0)	0 (0)	1 (2.6)	-	-
	No	16 (100)	45 (100)	37 (97.4)		
Coronavirus OC43	Yes	0 (0)	0 (0)	2 (5.3)	-	-
	No	16 (100)	45 (100)	36 (94.7)		
Influenza A	Yes	0 (0)	1 (2.2)	1 (2.6)	1.62 [0.10 - 26.72], P = 0.74	-

	No	16 (100)	44 (97.8)	37 (97.4)		
At least one virus	Yes	5 (31.3)	19 (42.2)	22 (57.9)	2.11 [0.93 - 4.83], P = 0.07	2.14 [0.69 – 6.72], P = 0.19
	No	11 (68.7)	26 (57.8)	16 (42.1)		

*at least one respiratory symptom (cough, sore throat, rhinitis)

OR: odds ratio, CI: confidence interval

Table 5. Frequency of pathogens in post-Hajj samples according to influenza-like illness symptoms at the time of sampling.

		No ILI* (N = 75)	Resolved ILI* (N = 12)	Ongoing ILI* (N = 12)	OR [95%CI], P value (ongoing ILI versus no ILI or resolved ILI)	OR [95%CI], P value (ongoing or resolved ILI versus no ILI)
Rhinovirus/enterovirus	Yes	23 (30.7)	3 (25.0)	6 (50.0)	2.34 [0.70-7.96], P = 0.17	1.35 [0.52 – 3.55], P = 0.53
	No	52 (69.3)	9 (75.0)	6 (50.0)		
Adenovirus	Yes	3 (4.0)	0 (0)	0 (0)	-	-
	No	72 (96.0)	12 (100)	12 (100)		
Coronavirus 229E	Yes	10 (13.3)	1 (8.3)	4 (33.3)	3.45 [0.89 - 13.41], P = 0.07	1.71 [0.52 – 5.62], P = 0.38
	No	65 (86.7)	11 (91.7)	8 (66.7)		
Coronavirus HKU1	Yes	0 (0)	0 (0)	1 (8.3)	-	-
	No	75 (100)	12 (100)	11 (91.7)		
Coronavirus NL63	Yes	1 (1.3)	0 (0)	0 (0)	-	-
	No	74 (98.7)	12 (100)	12 (100)		
Coronavirus OC43	Yes	2 (2.7)	0 (0)	0 (0)	-	-
	No	73 (97.3)	12 (100)	12 (100)		
Influenza A	Yes	2 (2.7)	0 (0)	0 (0)	-	-

	No	73 (97.3)	12 (100)	12 (100)		
At least one virus	Yes	33 (44.0)	4 (33.3)	9 (75.0)	4.05 [1.02 - 16.02], P = 0.05	1.50 [0.59 – 3.79], P = 0.39
	No	42 (56.0)	8 (66.7)	3 (25.0)		

*ILI: influenza-like illness (cough, sore throat and fever)

OR: Odds ratio, CI: confidence interval