



BMJ Open Multiyear prospective cohort study to evaluate the risk potential of MERS-CoV infection among Malaysian Hajj pilgrims (MERCURIAL): a study protocol

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To cite: Johari J, Hontz RD, Pike BL, *et al.* Multiyear prospective cohort study to evaluate the risk potential of MERS-CoV infection among Malaysian Hajj pilgrims (MERCURIAL): a study protocol. *BMJ Open* 2021;**11**:e050901. doi:10.1136/bmjopen-2021-050901

► Prepublication history for this paper is available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2021-050901>).

Received 03 March 2021
Accepted 09 August 2021



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ABSTRACT

Introduction Middle East respiratory syndrome (MERS) is a viral respiratory infection caused by the MERS-CoV. MERS was first reported in the Kingdom of Saudi Arabia in 2012. Every year, the Hajj pilgrimage to Mecca attracts more than two million pilgrims from 184 countries, making it one of the largest annual religious mass gatherings (MGs) worldwide. MGs in confined areas with a high number of pilgrims' movements worldwide continues to elicit significant global public health concerns. MERCURIAL was designed by adopting a seroconversion surveillance approach to provide multiyear evidence of MG-associated MERS-CoV seroconversion among the Malaysian Hajj pilgrims.

Methods and analysis MERCURIAL is an ongoing multiyear prospective cohort study. Every year, for the next 5 years, a cohort of 1000 Hajj pilgrims was enrolled beginning in the 2016 Hajj pilgrimage season. Pre-Hajj and post-Hajj serum samples were obtained and serologically analysed for evidence of MERS-CoV seroconversion. Sociodemographic data, underlying medical conditions, symptoms experienced during Hajj pilgrimage, and exposure to camel and untreated camel products were recorded using structured pre-Hajj and post-Hajj questionnaires. The possible risk factors associated with the seroconversion data were analysed using univariate and multivariate logistic regression. The primary outcome of this study is to better enhance our understanding of the potential threat of MERS-CoV spreading through MG beyond the Middle East.

Ethics and dissemination This study has obtained ethical approval from the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia. Results from the study will be submitted for publication in peer-reviewed journals and presented in conferences and scientific meetings.

Strengths and limitations of this study

- MERCURIAL will serve as a platform for the first multiyear seroconversion surveillance study assessing mass gathering-associated Middle East respiratory syndrome (MERS)-CoV seroconversion among Hajj pilgrims travelling to the regions that are most impacted by this virus.
- Our prospective study design will allow us to look at antibody titre changes in pre-Hajj and post-Hajj serum samples in the same individuals, providing serological evidence of exposure to MERS-CoV.
- This study will produce evidence that may influence future healthcare services for Hajj pilgrims in Malaysia and other countries worldwide.
- The drop-out rate may be high due to the limited time frame and other challenges we may face during post-Hajj follow-up activities at the airport.
- As with any cohort study, there is potential for response bias.

Trial registration number NMRR-15-1640-25391.

INTRODUCTION

Middle East respiratory syndrome (MERS) is a viral respiratory disease that first emerged in the Kingdom of Saudi Arabia (KSA) in 2012, caused by a novel coronavirus, MERS-CoV.¹ It is claimed to have been transmitted from animals to humans, most likely through direct or indirect contact with infected dromedary camels, which was found to be a potential reservoir host for MERS-CoV.²



Human-to-human transmission is rare, but most cases have occurred in healthcare settings due to close contact when providing care to infected patients.³ The person who contracted the virus can be asymptomatic or present with mild symptoms such as fever, cough, shortness of breath and diarrhoea. Some people, however, develop a severe acute respiratory illness that can lead to death. Among those who succumbed to the infection, many were among the elderly and those with underlying medical problems such as cancer, cardiovascular diseases, diabetes and chronic lung disease.⁴ MERS has emerged as a serious global public health concern due to its high mortality rate with the potential to spread beyond the Middle East. By 2020, more than 2500 MERS-CoV laboratory-confirmed cases, resulting in more than 800 associated deaths has been reported globally. To date, the most significant MERS outbreak outside of the Middle East occurred in the Republic of Korea.⁵ Travel history to the Middle East was identified as the source of infection reported outside of the region.⁶

The potential of MERS to spread through mass gatherings (MGs) is of significant public health concern as it involves a high concentration of people at a specific location over a set period, hence, increasing the chance of transmission.⁷ Every year, KSA hosts the Hajj pilgrimage, attracting more than two million pilgrims from at least 184 countries to the city of Mecca.⁸ This substantial MG creates a potential high-risk condition for the global spread of MERS due to high population mobility post-Hajj. Moreover, a majority of pilgrims are among the elderly with comorbidities such as diabetes and hypertension, putting them in the high-risk group with increased susceptibility to respiratory tract infections.⁹

Pandemic potential of this disease serves as an impetus for some epidemiological studies related to Hajj pilgrims and MERS-CoV from researchers worldwide.¹⁰ However, the availability of MG-associated surveillance data is limited to the existing studies focusing primarily on detecting active infection in symptomatic Hajj pilgrims using a standard reverse transcription polymerase chain reaction (RT-PCR) molecular diagnostic assay. To date, no confirmed cases have been detected in Hajj pilgrims using RT-PCR,^{11–23} imploring an enhanced surveillance system to detect ‘silent’ disease transmission among Hajj pilgrims especially in those with mild and asymptomatic infection.

This study protocol outlines a multiyear prospective cohort study among Malaysian Hajj pilgrims by integrating seroconversion surveillance to assess MG-associated MERS-CoV seroconversion during the Hajj pilgrimage. The coordinated and structured manner of the handling of Malaysian Hajj pilgrims by the Malaysian Hajj Pilgrim Fund Board (Tabung Haji Malaysia, THM) and the long-standing participation of the Ministry of Health Malaysia (MOHM) in the management of the Malaysian Hajj pilgrims offered an excellent opportunity to undertake this study.

OBJECTIVES

Primary objective

The primary objective of this study is to estimate the MERS-CoV seroconversion rate during the Hajj pilgrimage.

Secondary objectives

Specifically, this study has three secondary objectives:

1. To determine, as measured by seroconversion, the risk of exposure to MERS-CoV among Malaysian pilgrims travelling to/from Saudi Arabia for Hajj.
2. To ascertain the percentage of exposed MERS-CoV individuals that go unreported due to a lack of symptoms or mild clinical presentations.
3. To describe the risk factors (age, gender, exposure to camels or untreated camel products, etc) associated with seroconversion to MERS-CoV within the described Hajj pilgrimage cohort.

METHODS AND ANALYSIS

Study design

This is a multiyear prospective cohort study designed to evaluate the risk potential of MERS-CoV infection among the Malaysian Hajj pilgrims from different cohorts beginning with the 2016 Hajj pilgrimage season using the seroconversion surveillance methodology. This study was initiated in 2016 and is expected to continue for another 5 years through 2021. The quantitative data will consist of both biological samples and survey questionnaires. The biological samples include paired-serum samples (pre-Hajj serum and post-Hajj serum) to identify the presence of anti-MERS-CoV antibodies among the pilgrims. The pre-Hajj and post-Hajj questionnaires are structured to provide data on the health status and the history of exposures to camels and untreated camel products during the pilgrimage. These questionnaires are compiled based on the sample questionnaire originating from a WHO protocol aimed to assess potential risk factors associated with the introduction or transmission of MERS-CoV.²⁴ The schematic of the overall study is as presented in [figure 1](#).

Study team

The study team was composed of researchers from the Tropical Infectious Diseases Research & Education Centre (TIDREC) at the University of Malaya, and the US Naval Medical Research Center-Asia (NMRC-A) in Singapore, with key management personnel from the MOHM and THM. The researchers from TIDREC and NMRC-A were responsible for planning, coordinating, and ensuring that ethical standards were maintained throughout the study. Key management personnel from MOHM and THM participated in their official capacities and authority to directly or indirectly planned, directed and managed healthcare activities concerning the Malaysian Hajj pilgrims. The microneutralisation assay protocols were developed and transferred to TIDREC by research partners at the NMRC in Silver Spring, Maryland, USA.

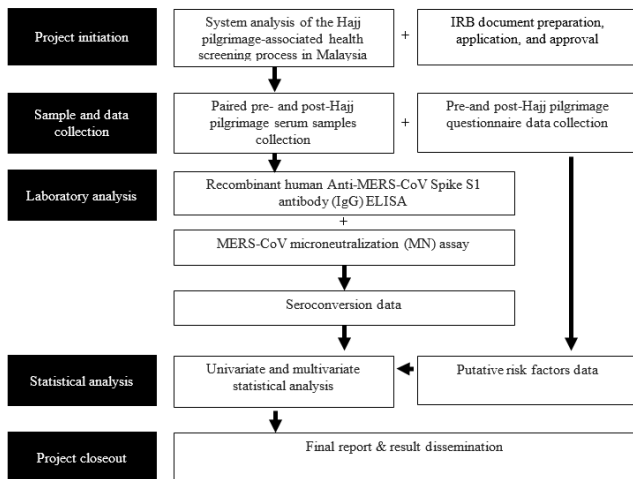


Figure 1 The schematic flow chart of the overall implementation of MERCURIAL. IRB, Institutional Review Board; MERS, Middle East respiratory syndrome.

Patient and public involvement

It was not appropriate or possible to involve patients or the public in the design, or conduct, or reporting, or dissemination plans of our research.

Study population

The study population consists of Malaysian Hajj pilgrims selected by THM to perform the pilgrimage beginning from the 2016 Hajj pilgrimage season, aged from 18 years and above and are residing in the area of Greater Kuala Lumpur metropolitan area (ie, Kuala Lumpur, Putrajaya and Selangor) (figure 2). The eligibility criteria for the study participants are as follows:

Inclusion criteria

1. Attended the pre-Hajj health screening conducted by MOHM and THM.
2. ≥ 18 years of age and completed the informed consent.

Exclusion criteria

1. Participants who do not fulfil the criteria during the pre-Hajj health screening conducted by MOHM and THM.

Study sites

Enrolment of study participants and sampling of the pre-Hajj serum were conducted at six MOHM health facilities

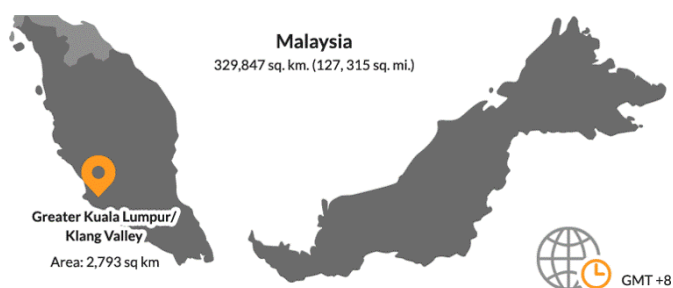


Figure 2 Map of location of the greater Kuala Lumpur metropolitan areas marked by orange pin.



Figure 3 Map of locations of six pre-Hajj enrolment sites marked by orange dots and post-Hajj follow-up site at the Kuala Lumpur International Airport, marked by the blue dot. KL, Kuala Lumpur.

in the Greater Kuala Lumpur metropolitan area and, with few instances, were undertaken at selected THM branch offices. The post-Hajj follow-up and serum sampling were performed at the Arrival Lounge of the Kuala Lumpur International Airport (KLIA), following Hajj pilgrim disembarkment or during in-transit waiting. Figure 3 shows the study locations for pre-Hajj enrolment and post-Hajj follow-up activities.

Sample size calculation

Calculated sample size ($n=1000$) was determined based on the assumption that 1.0% of returned pilgrims from the Hajj will demonstrate evidence of exposure to MERS-CoV (ie, positive seroconversion results). Hence, to have a 95% probability of observing five seroconversions; 914 individuals would need to be enrolled into each annual cohort which also includes, at most, 86 potential dropouts per year taking into consideration that investigators were unable to meet the individuals during the follow-ups at the airport.

Sample and data collection

Pre-Hajj enrolment

The pre-Hajj enrolments were carried out during routine health screenings of the prospective Malaysian Hajj pilgrims organised by MOHM and THM with at least five study personnel stationed at each selected study site. Bunting and advertising the study were placed to facilitate enrolment. When the prospective pilgrims arrived at any of the study sites, those who were interested to volunteer for the study were briefed on the study scope, objectives and procedures involved. Written informed consent was obtained from all eligible study participants before any screening and enrolment procedures took place. A pre-Hajj questionnaire assessing study participants' demographic data, general health information and travel history was administered. Then 5 mL of pre-Hajj blood sample (baseline sample) was obtained from each participant. Blood was collected into a serum-separating tube and labelled with a coded identification number.

A similar identifier was also recorded on the pre-Hajj questionnaire. Serum samples were handled according to standard biosafety measures and transported to the laboratory on the same day and stored at -20°C before analysis. Enrolled study participants were provided with a study sticker placed onto their Hajj pilgrim medical record booklet to facilitate participant's identification during the follow-ups. The study participants were also briefed about the follow-up process that will take place on their arrival back from Hajj.

Post-Hajj follow-up

Details of the return flights of study participants were provided by THM. In order to limit lost to follow-up, a reminder memo was sent to every study participant just before they departed from KSA. On arrival at the KLIA Arrival Lounge, placards were displayed to assist study participants in the due process. With collaboration between the KLIA Health Office and THM, a study follow-up station was set up at the KLIA Arrival Lounge, with at least ten study personnel on standby. Security clearance for each study personnel was obtained from the KLIA Security Office. Study personnel received seasonal influenza and hepatitis B vaccines and required to strictly adhere to all the biosafety practices (ie, use of proper personal protective equipment and following standard operating procedures) throughout the sampling activities.

Hajj pilgrims had to pass through a thermal scanner set up by the MOHM officers on arrival at the KLIA. Participants with body temperature of 38°C and above were directed to dedicated booths for further screening. Meanwhile, healthy study participants were directed to the designated study stations by the staff on duty. A post-pilgrimage questionnaire was administered to obtain data on any respiratory symptoms experienced throughout the Hajj pilgrimage. Additionally, information on visits to camel farms, and exposure to camels and untreated camel products were obtained. A second blood sample (5 mL of follow-up sample) was obtained in a serum-separating tube by a phlebotomist on-site. Serum samples were packaged, labelled and transported in iceboxes to TIDREC on collection day and stored at -20°C until further analysis.

Laboratory analysis

Paired pre-pilgrimage and post-pilgrimage serum samples were tested for the presence of MERS-CoV-specific antibodies using commercially available anti-MERS-CoV IgG rS1 ELISA kit (EUROIMMUN, Lübeck, Germany) by strictly following the manufacturer's recommendations. The preliminary cut-off value was calculated as mean optical density (OD) $+3$ SDs from at least 1000 seronegative serum samples. Serum samples with OD above the cut-off values were recorded as seropositive. Seroconversion, or less than fourfold increase in MERS-CoV-specific IgG titre in post-Hajj serum samples among the initially seronegative samples, was considered suggestive of a recent exposure to MERS-CoV infection.

An assay for detecting neutralising antibodies against MERS-CoV was performed on the seropositive serum samples using the confirmatory microneutralisation test developed in-house by NMRC. Briefly, serum samples were heat inactivated and serially diluted. Diluted serum samples were then mixed with live MERS-CoV virus prior to incubation with Vero cells. Following a 2-day incubation period, cells were fixed and analysed by quantitative ELISA. Viral neutralisation for the dilutions were considered when the OD values for the ELISAs are at least 50% of the OD values of the virus control. The microneutralisation assay was entirely performed in the biosafety level 3 containment facility at TIDREC.

Data analysis

All questionnaire data were coded, cleaned and transferred into the statistical SPSS software V.25 (IBM). Study population characteristics were analysed and summarised using descriptive statistics. Combined with serological data, putative risk factors were identified using univariate analysis. Statistical analyses were focused on MERS-CoV seroconversions and associations with relevant, available independent variables such as age, gender, comorbidities, healthcare visits and environmental exposures to camels and untreated camel products. Risk factors correlated with seroconversion were further investigated in a multivariable logistics analysis and adjusted for confounders.

CURRENT AND FUTURE DIRECTIONS

The emergence of three highly contagious and deadly human coronaviruses, namely, SARS-CoV, MERS-CoV and the most recent SARS-CoV-2 (COVID-19) pandemic over the past 20 years has sparked public health concerns and global economic crisis, although no cases of SARS-CoV reported for over a decade.²⁵ However, there have been greater concerns of co-circulation of SARS-CoV-2 with MERS-CoV in the KSA given the potential spread beyond the Middle East following Hajj pilgrimage during the largest annual MG in the world.^{26 27}

Importantly, previous attempts to detect MERS-CoV infection among returning Hajj pilgrims using RT-PCR in several countries have yet to report any confirmed cases.¹¹⁻²³ MERCURIAL was designed to offer an enhanced surveillance system by incorporating a serological assay to detect seroconversion among Hajj pilgrims. To achieve this, MERCURIAL leveraged the existing health screening infrastructure in Malaysia to collect pre-Hajj and post-Hajj serum samples from Hajj pilgrims along with detailed questionnaire data. Moreover, the serological assay has a clear advantage over RT-PCR in that the antibodies stay for longer periods following infection as compared with the pathogen and even if the viruses are mutated, the antigenic similarity is essentially retained, making it a better biomarker for surveillance.^{28 29} Moreover, MERS-CoV contains proteins S (including subunits S1 and S2) and N that are highly immunogenic, which can provoke immune response, making it a good candidate for developing immunoassays to detect

virus-specific antibodies.³⁰ The seroconversion surveillance approach such as MERCURIAL will further enhance our understanding and preparedness of emerging infections, as seroepidemiology data able to estimate the burden of the diseases, predict future outbreaks and identify high-risk groups as well as formulate vaccine policy and healthcare resources.³¹

However, there are limitations expected from this study that could be addressed in future research. The primary limitation is the timing of the post-Hajj blood sampling on pilgrim's arrival from Hajj, which may fall within the viral incubation window period where antibody rise may not yet be detectable by serological tests. This is compounded by the Institutional Review Board (IRB) approval, which does not allow for a post-arrival follow-up of the participants. These limitations may result in an underestimation of the actual seroconversion rates. Ideally, future cohorts should consider allowing a week-4 follow-up post-Hajj, or through pilgrims' self-reporting to the nearest public health clinics to provide better evidence for disease exposure. Additionally, the inability to follow up with the study participants while in the KSA during the Hajj season may lead to missing data points regarding the exact exposure time and the possible contributing factors. Hence, to minimise this, a post-pilgrimage questionnaire will be given, which consists of the collection of data on any respiratory symptoms experienced during the Hajj period. We anticipate new collaboration opportunities with technology providers, particularly on the use of wearable temperature sensor wristbands for constant and real-time monitoring of body temperatures of participants throughout the pilgrimage in future cohorts to render more precise and comprehensive data collection.

Another potential limitation in the study is the study area that covered only Hajj pilgrims from the more urbanised Greater Kuala Lumpur metropolitan area where inhabitants are likely to be more educated. Therefore, these people may have lower risk than those from more rural states who are elderly, and are more likely to have underlying comorbidities. Future cohorts should include Hajj pilgrims from other parts of the country as well, if resources permit, in order to better represent the general Malaysian population.

Since its inception, the MERCURIAL Biological Samples and Data Repository comprises a collection of paired serum samples linked to corresponding questionnaire data from at least three different Malaysian Hajj pilgrims' cohorts. The emergence of COVID-19 in late 2019 led to shutdown of most of the affected countries' international borders, including the KSA and Malaysia, posing several interruptions in MERCURIAL due processes. At present, while the study team is anticipating the reopening of Mecca for Hajj pilgrims from outside the KSA, the possibilities to expand MERCURIAL to include the seroconversion surveillance for COVID-19 are being explored. The establishment of a long-term cohort study such as this could be a valuable tool in the surveillance and monitoring of the movement of any potential emerging pathogens in the future.

Ethics and dissemination

This study has obtained ethical approval from the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia, in compliance with all applicable federal regulations governing the protection of human subjects. This work was completed under HRPO.2017.0005 to NMRC-Asia. Findings from the study will be disseminated through conference presentations and publications in peer-reviewed journals. Results will also be disseminated through annual Malaysian Hajj Pilgrims Health Services Technical Committee Meeting organised by MOHM and THM.

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Acknowledgements The authors thank all staff and officers from the MOHM and THM for their valuable contributions in this study. The authors also would like to thank the Director General of Health Malaysia for his permission to publish this article.

Contributors SA, BLP, C-KC, NR, L-HS, KV, RMZ, JAG-R, TH and JJ were involved in designing the study protocol. JJ, A-SA, CSK, S-SN, VT, HYL, B-TT, SSS, J-JK, JAJ, S-KL, CNY, N-HM, NSA, NAACMS, N-FM-R, HH and HY were involved in the conduct of the study and data collection. JJ, SA and RDH were responsible in preparing the manuscript. All authors were involved in critical revision of the manuscript for intellectual content.

Funding The study was supported by the US Naval Medical Research Centre-Asia (NMRC-A) through funds received from the US Department of Defence's Global Emerging Infections Surveillance (DoD-GEIS) programme and the Defence Threat Reduction Agency (DTRA)/Cooperative Biological Engagement Programme (CBEP). This work was supported/funded by work unit number D1604.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

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