Cross-sectional seroepidemiologic study of MERS-CoV infection in high risk populations in contact with dromedary camels

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PROTOCOL SUMMARY

Representative serologic studies are designed to collect data to estimate prevalence of antibodies to a new pathogen in a population. This information is critical to better understand the extent of infection in a population and the severity of the new virus.

This study protocol outlines methods to collect data to measure the seroprevalence of cross-reactive antibodies to MERS-CoV in presumed high-risk human populations and to detect viral shedding in dromedary camels in selected countries with high densities of dromedary camel populations.

The study focuses on occupational risk - individuals whose work requires direct and prolonged contact with dromedary camels - and has been adapted for consideration in African and South Asian countries.

The original protocol was published in 2013 and has been updated to reflect updated scientific knowledge about MERS-CoV, the results and experiences of similar studies conducted in a number of countries and input from the FAO and the animal sector.

Comments for the user's consideration are provided in purple text throughout the document as the user may need to modify methods slightly because of the local context in which this study will be carried out.

TABLE OF CONTENTS

PROTOCOL SUMMARY 3
1.0 SCIENTIFIC BACKGROUND AND RATIONALE 5
1.1 STUDY OBJECTIVES 5
1.1.1 PRIMARY OBJECTIVES 5
2.0 STUDY PROCEDURES 7
2.1 SELECTION AND RECRUITMENT OF STUDY PARTICIPANTS 7
2.1.1 IDENTIFICATION OF STUDY PARTICIPANTS - SELECTION OF HIGH RISK POPULATIONS 7
2.1.2 ELIGIBILITY CRITERIA 8
2.1.3 ETHICAL CONSIDERATIONS 8
2.1.4 INFORMED CONSENT 9
2.1.5 RISKS AND BENEFITS FOR PARTICIPANTS 9
2.1.6 COMPENSATION AND INCENTIVES TO PARTICIPATE 9
2.1.7 PREVENTION OF MERS-COV INFECTION 9
2.1.8 DATA COLLECTION 9
2.2 SPECIMEN COLLECTION AND LABORATORY EVALUATIONS 9
2.2.1 SPECIMEN COLLECTION, TRANSPORTATION 9
2.2.2 LABORATORY EVALUATIONS 10
2.3 DATA MANAGEMENT 12
3.0 STUDY ENDPOINTS & STATISTICAL ANALYSIS 13
3.1 SAMPLE SIZE CONSIDERATIONS 13
3.2 STUDY OUTCOME MEASURES 13
3.2.1 PRIMARY ENDPOINTS 13
4.0 COMPOSITION OF STUDY TEAM 14
5.0 DISSEMINATION OF RESULTS 15
ACKNOWLEDGEMENTS 16
REFERENCES 17
APPENDICES 19
APPENDIX A: QUESTIONNAIRES FOR OCCUPATIONALLY EXPOSED PARTICIPANTS 19
APPENDIX B: POPULATION SPECIFIC QUESTIONNAIRES USED IN QATAR 20

1.0 SCIENTIFIC BACKGROUND AND RATIONALE

As of June 2018, more than 2200 laboratory-confirmed cases of human infection with Middle East Respiratory syndrome coronavirus (MERS-CoV) have been reported to WHO [1]. The virus appears to be circulating widely in dromedary camel populations throughout the Middle East, in large parts of Africa and in South Asia. The majority of human cases have been reported from the Kingdom of Saudi Arabia.

MERS-CoV is a zoonotic virus and dromedary camels are the single maintenance host and primary reservoir of MERS-CoV, however, the route of transmission to humans is unknown [2,3]. MERS-CoV nucleic acid has been identified in camels in Egypt, Oman, Qatar, United Arab Emirates, and Saudi Arabia and sera from dromedary camels from a number of (18) countries throughout the Middle East, North, West and East Africa and Pakistan have been found to have antibodies to MERS-CoV [4-11]. Human-to-human transmission has occurred in several clusters in health care facilities, most notably in March and April of 2014 in Saudi Arabia and the United Arab Emirates, as well as in 2015 in the Republic of Korea where 186 cases resulted from a single imported case. There is evidence of limited transmission among family members in households and in one work setting [1,2,12-16]. A number of asymptomatic or mild MERS-CoV cases have been identified via contact tracing but as surveillance targets patients with severe disease, the proportion of infections that may be mild or asymptomatic is unclear. The secondary attack rate in families and other social groups appears to be low.

The non-specificity of clinical case definitions for MERS-CoV and the tendency of surveillance to focus on severe cases mean that rates of infection cannot be estimated from case-based clinical surveillance. Yet, this information is critical to understanding the overall morbidity, mortality, and population-level severity of a novel virus. Representative serological studies are designed to collect denominator data (i.e., number of infections) that can be used to estimate severity parameters such as the case fatality risk (i.e. the total number of novel virus-related deaths divided by the total number of infections) and hospitalization risk (number of related hospitalizations divided by number of infections). Thus analysis of serological data can reduce the uncertainty around severity assessment and help inform the appropriate intensity and targeting of mitigation policies.

In the study protocol outlined here, we provide the methods to conduct a cross-sectional sero-epidemiologic study of populations that are believed to be at a higher risk of infection due to prolonged direct contact with dromedary camels (e.g., camel workers, camel handlers, camel slaughterhouse workers), be conducted to estimate seroprevalence of MERS-CoV in specific populations at one point in time. This information will be useful to better understand the risk of infection among people who are likely to be more frequently exposed to suspected animal reservoirs.

1.1 STUDY OBJECTIVES

The data collected from this study can be used to refine/update recommendations for surveillance and case definitions, to characterize the key epidemiological transmission features of MERS-CoV virus, help understand spread, severity, spectrum of disease, impact on the community and to inform operational models for implementation of countermeasures such as case isolation, contact tracing and isolation.

1.1.1 PRIMARY OBJECTIVES

The primary objectives of this study are to:

- To determine the geographic extent of evidence of MERS-CoV viral shedding in dromedary camels in countries with high densities of dromedary camels

- To determine the geographic extent of evidence of MERS-CoV infection in occupationally exposed persons in countries with high densities of dromedary camels
- Estimate seroprevalence of MERS-CoV infections among persons with occupational exposure to dromedary camels

COMMENT: Seroepidemiology studies, such as the one described here, provide rich data that can permit evaluation of numerous secondary objectives. These can include, but not limited to:

- Identify modifiable risk factors (e.g. exposures, behaviors, practices) for human MERS-CoV infection among persons with occupational exposure to dromedary camels

2.0 STUDY PROCEDURES

Because MERS-CoV has not yet achieved sustained human-to-human transmission in the population, this study utilizes a cross-sectional design, i.e., the study of a population at one point in time, rather than a serial cross-sectional study in which sera were collected from two time periods (pre and post) in which cumulative incidence could be estimated (such as is done for influenza).

2.1 SELECTION AND RECRUITMENT OF STUDY PARTICIPANTS

There are several options for the selection of study participants and these are described below.

COMMENT: For MERS-CoV, we recommend that study participants be chosen from populations in regular direct contact with dromedary camels (e.g. dromedary camel handlers on farms, in live animal markets, at abattoirs, etc.). The exact locations where study participants will be recruited will need to be determined by the camel husbandry and slaughterhouse practices of each country.

2.1.1 IDENTIFICATION OF STUDY PARTICIPANTS - SELECTION OF HIGH RISK POPULATIONS

COMMENT: The methods for sampling will depend on the study population selected. Below are examples of some populations that may be considered for inclusion in this study. The specific methods for selection and recruitment will depend on the populations selected and the locations of these populations.

Recruitment of participants from camel farms

- Identify the locations of the highest density of domesticated dromedary camel farms/dromedary camels in the country

COMMENT: It is recommended that investigators identify geographic areas/sites with the largest number of potential participants for this study and attempt to recruit all available participants.

COMMENT: If the farm population is small, then all eligible study participants should be recruited. If not, then a random selection of eligible residents and workers of the farm should be selected.

- At each camel farm, the potential study participant will first be asked whether he/she is over the age of [local age of consent]. If the respondent is not, the interviewer will ask to speak with an individual who is over the local age of consent. The interviewer will ask the potential study participant how many individuals are living and working at the farm who are over the age of consent, to name them, and who are present. A random name will be chosen from those present and interviewers will ask to speak to each participant at the farm. The study will be briefly described to the responding participant or guardian and a time arranged to return and formally recruit them to the study.

If no one is at home at the first visit, each randomly selected farm will be visited 2 additional times at different times of the day and each visit recorded.

Recruitment of participants from animal markets

- The locations of dromedary camel markets in the country will be identified.
- If there are a large number of markets within the participating country, markets from provinces where MERS-CoV human cases have been confirmed can be randomly selected. In countries that have not reported any MERS-CoV human infections, markets in close proximity to dromedary

camel farms/ dromedary camel populations can be selected. From each market, a random selection of eligible market workers will be recruited for the study.

COMMENT: If MERS-CoV has not been detected in the implementing country, then a random selection of camel markets can be chosen. If MERS-CoV has been detected, it is preferable to focus on markets in the area(s) where the virus has been detected.

- For eligible markets, study personnel will visit the market to identify the number of market workers eligible for inclusion and obtain relevant approvals to conduct this study in the market. If the markets are small in size, study personnel will attempt to recruit all eligible market workers into the study. If the markets are large in size, study personnel will select a random sample of market workers to include in the study until the sample size is met.

Recruitment of participants from animal handlers from racing venues (e.g., camel racing tracks)

- The locations of dromedary camel racing venues in the country will be identified.
- For eligible venues, study personnel will visit the venue to identify the number of workers eligible for inclusion. If the venue is small in size, study personnel will attempt to recruit all eligible workers into the study. If the venue is large in size, study personnel will select a random sample of workers to include in the study until the sample size is met.

Recruitment of participants from camel abattoirs

- The locations of camel abattoirs in the country will be identified.
- Camel abattoirs from provinces with the highest volume of camels processed will be selected.
- For eligible abattoirs, study personnel will visit the abattoir to identify the number of workers eligible for inclusion and obtain relevant approvals to conduct this study in the abattoir. If the abattoir is small in size, study personnel will attempt to recruit all eligible abattoir workers and animal handlers into the study. If the abattoir is large in size, study personnel will select a random sample of abattoir workers and animal handlers to include in the study until the sample size is met.

For occupational study populations, it will not be possible to obtain sufficient samples in all age groups. However, efforts should be made to include participants that are representative of all job-types within the occupational cohort of interest.

For all four of the selection methods described, both the number of people that are requested to participate and the number that refuse will need to be recorded.

2.1.2 ELIGIBILITY CRITERIA

Inclusion criteria: any individual 18 years old or above, who gives informed consent.

COMMENT: The age of consent and assent may vary by country. Check with local IRB requirements.

Exclusion criteria: any contraindication to venipuncture.

2.1.3 ETHICAL CONSIDERATIONS

Ethical approval will be sought in accordance with local, regional and national authorities.

2.1.4 INFORMED CONSENT

The purpose of the investigation will be explained to all eligible participants. Informed consent will be sought from all eligible study participants willing to participate in the investigation by a trained member of the investigation team before any procedure is performed as part of the investigation. Each participant must be informed that participation in the investigation is voluntary and that s/he is free to withdraw, without justification, from the investigation at any time without consequences.

Informed consent will seek approval to collect demographic data, information related to exposures to dromedary camels, and a blood and nasopharyengeal sample to determine exposure to MERS-CoV. Informed consent will indicate that samples may be shipped outside of the home country for additional testing and that samples may be used for future research purposes. Informed consent will also indicate that any suspected or confirmed MERS-CoV infection may be notified to the national health authorities under the requirements of the International Health Regulations.

2.1.5 RISKS AND BENEFITS FOR PARTICIPANTS

This investigation poses minimal risk to participants, involving the collection of a small amount of blood. The direct benefit to the participant is the possibility of identifying evidence of MERS-CoV infection, which, if the infection were acute, would allow for early monitoring and treatment. Additional benefits are indirect in that data collected will help improve efforts to understand the geographic scope and transmission of MERS-CoV, assisting efforts to reduce further spread of MERS-CoV.

2.1.6 COMPENSATION AND INCENTIVES TO PARTICIPATE

COMMENT: If local IRB regulations permit, participants may be offered reimbursement for reasonable out of pocket expenses related to the investigation; however, the level of compensation should not be such that participants are unduly influenced into consenting to participate.

2.1.7 PREVENTION OF MERS-COV INFECTION

Before the start of the investigation, all study personnel, will be offered training in local infection prevention and control procedures (standard contact, droplet or airborne precautions). These procedures will be determined by local or national guidelines, but should include proper hand hygiene and the correct use of surgical or respiratory face masks, if necessary, to minimize the risk of infection and transmission. WHO technical guidance on infection prevention and control specific to MERS-CoV can be found here:

http://www.who.int/csr/disease/coronavirus_infections/technical-guidance-infection/en/

2.1.8 DATA COLLECTION

At the time of recruitment, a study questionnaire will be administered to all study participants, depending on the occupation of the participant. Appendix A provides examples of questionnaires that may be used for camel farm workers, live animal market workers, abattoir workers and quarantine workers.

COMMENT: Appendix B contains population specific questionnaires used for a similar study conducted in Qatar in 2014.

2.2 SPECIMEN COLLECTION AND LABORATORY EVALUATIONS

2.2.1 SPECIMEN COLLECTION, TRANSPORTATION

All participants: 5-10 mL of blood will be collected in a serum tube according to standard procedures and labeled with a coded identification number that will also be recorded on the interview questionnaire. All

participants will also have a nasopharyngeal and oropharyngeal swab collected and tested for MERS-CoV by RT-PCR. Time of collection, location, and name of person collecting the specimens will also be recorded. Specimen tubes will be stored temporarily on ice carried by the study teams until they can be transported to the laboratory.

Transport of specimens within national borders should comply with applicable national regulations. International transport of MERS-CoV specimens should follow applicable international regulations as described in the WHO Guidance on Regulations for the Transport of Infectious Substances 2013- 2014 available at: http://www.who.int/ihr/publications/whohseihr20100801/en/index.html.

COMMENT: The specific volume of blood to be determined by study personnel, bearing in mind that the minimum required volume is 5 mL.

COMMENT: Specimens may be aliquotted so that specimens remain in country and only aliquots are sent to a reference lab. Some serologic assays may become available to be done in country.

Full details for virologic laboratory testing of MERS-CoV can be found here:

http://www.who.int/csr/disease/coronavirus_infections/technical-guidance-laboratory/en/

If any participants return a positive PCR test for MERS-CoV, they should be reported to the national health authorities under the requirements of the International Health Regulations, and all contacts followed up for 14 days.

See: http://www.who.int/csr/disease/coronavirus_infections/mers-investigation-cases/en/

Sampling in dromedary camels: Nasal samples from dromedary camels at the study sites (e.g., where human participants are recruited) will be collected at the time of the human biological sampling and tested for MERS-CoV by RT-PCR methods at the appropriate national animal health laboratory. Sampling of younger camels should be prioritized, particularly during the time of the year when young camels are weaned from their mothers.

COMMENT: This protocol recommends testing the dromedary camels or meat/organs/products (in the case of a slaughterhouse) to determine if there is active viral shedding at the study locations. Sampling will need to be conducted with the Ministry of Agriculture or relevant national/sub-national authorities as determined by each country.

2.2.2 LABORATORY EVALUATIONS

As of January 2018, a MERS-CoV case may be laboratory confirmed by detection of viral nucleic acid or by serology. WHO case definitions for MERS-CoV can be found here:

http://www.who.int/csr/disease/coronavirus_infections/case_definition/en/

COMMENT: The following laboratory recommendations are subject to further updates as diagnostic tests and approaches become available.

Molecular testing: Three rRT-PCR assays for routine detection of MERS-CoV have been developed and their details published. Currently described tests are an assay targeting upstream of the E protein gene (upE) and assays targeting the open reading frame 1b (ORF 1b) [9] and the open reading frame 1a (ORF 1a) [10]. The assay for the upE target is considered highly sensitive and is recommended for screening, with the ORF 1a

assay considered of equal sensitivity. The ORF 1b assay is considered less sensitive than the ORF 1a assay. An alternative approach involving two rRT-PCR assays targeting the MERS-CoV nucleocapsid (N) protein gene, which can complement upE and ORF 1a assays for screening and confirmation has also been published [11]. To date, these rRT-PCR assays have shown no cross-reactivity with other respiratory viruses including human coronaviruses and were suitable to detect all known MERS-CoV strains in humans and dromedary camels.

Methods for sequence confirmation have also been published [10].

Serologic testing: Serological testing will be carried out in collaboration with an external laboratory partner as needed. Multiple serological assays will be needed to confirm seropositivity, and may include fluorescent antibody testing, enzyme linked immunoassay, or luciferase assay. In addition, all samples will be tested using a neutralization assay. Testing will be done for antibodies against MERS-CoV specific proteins of the spike and nucleocapsid. Two aliquots of sample will be made and one kept for future analysis.

COMMENT: Several serological assays are currently available, having been developed by different laboratories using different platforms, including fluorescent antibodies, enzyme-linked immunosorbent assays, luciferase immunoprecipitation systems, and virus neutralization. The assays each have advantages and disadvantages but appear to have similar utility. Until their interoperability and comparability are better understood, more than one assay should be performed for each serum sample. An algorithm is being developed to determine the combinations of results considered "positive" for the purpose of comparative analysis.

A number of different technical approaches for confirming MERS-CoV infection using serology have been developed. Details of two immunofluorescence assays to detect antibodies to MERS-CoV have been published [10], and these assays, along with a serum neutralization test, were used in a 2 to 3 stage procedure to screen contacts of a case in Germany and determine population seroprevalences in KSA [9, 16,17]. An assay for detection of MERS-CoV antibodies using protein microarray technology has also been developed. Another two-stage approach with a screening test using a recombinant nucleocapsid (N) and spike (S) protein-based indirect enzyme-linked immunosorbent (ELISA), followed by a confirmatory microneutralization has also been described. Details of a neutralization test based on retroviral pseudoparticles which also demonstrates high levels of specificity to MERS-CoV have also been published [18].

COMMENT: Only a limited number of laboratories have the facilities for MERS-CoV serologic testing and therefore collaboration between countries without current capacity and designated reference laboratories is possible. Collaboration is at the discretion of Member States carrying out the investigation, but WHO strongly supports such collaboration and would willingly facilitate collaboration and possible shipment elsewhere for testing.

For serologic testing, if capacity for performing ELISA and/or microneutralization does not exist in country, WHO is able to facilitate coordination and collaboration with an external laboratory.

Sequencing: Full genome sequencing of the virus from biological samples from both human participants and dromedary camels should be performed, if possible. This may need to be done in collaboration with an external laboratory partner.

2.3 DATA MANAGEMENT

Demographic and occupational exposure data will be stored in a secure, password-protected database in the country where it is collected. Patient identity will be protected and only aggregate summary data released publically. Original data collection forms will be kept in locked storage.

3.0 STUDY ENDPOINTS & STATISTICAL ANALYSIS

The following section discusses sample size considerations, study endpoints – that is, what can be measured and calculated using the data collected in this study – and the statistical analyses that should be performed to answer the study questions.

3.1 SAMPLE SIZE CONSIDERATIONS

The study-specific sample size will be determined by the number of participants in contact with the dromedary camels and by assumptions related to MERS-CoV transmission from dromedary camels to humans.

COMMENT: Past studies have found it difficult to recruit large numbers of occupationally exposed individuals in individual countries. Every effort should be made to include all participants who are routinely in contact with dromedary camels as part of their occupational duties to maximize the statistical power of the investigation.

3.2 STUDY OUTCOME MEASURES

3.2.1 PRIMARY ENDPOINTS

The primary objectives of this study are to determine the geographic extent of evidence of MERS-CoV viral shedding in dromedary camels in countries with high density of dromedary camels; to determine the geographic extent of evidence of MERS-CoV infection in occupationally exposed persons in countries with high density of dromedary camels; and to estimate seroprevalence of MERS-CoV infections among persons with occupational exposure to dromedary camels.

The primary endpoints will therefore be:

- Proportion of all dromedary camels tested with evidence of MERS-CoV viral shedding
- Proportion of all persons with occupational exposure to dromedary camels having evidence of MERS-CoV infection
- Seroprevalence of MERS-CoV antibodies in human participants

COMMENT: Depending on the study sample size, these proportions may be reported as overall proportions or by subgroup (e.g. by occupational group or job duty, by age, gender, etc.).

4.0 COMPOSITION OF STUDY TEAM

COMMENT: The proposal calls for a multi-disciplinary research study team to undertake this study. The composition of the study team will be determined by each country. It is recommended that members from the Ministries of Health and Agriculture, national laboratories for animals and humans, and other partners are included in the implementation and interpretation of this investigation.

COMMENT: Once a study team is identified, a workshop and training should be conducted to organize the implementation of the study.

COMMENT: An advisory committee may be considered, as this study is part of a wider effort to conduct a multi-site cross-sectional human and dromedary camel serosurveys across many countries in Africa. This can further be discussed with WHO and partners.

5.0 DISSEMINATION OF RESULTS

Reports of the results of this study should include the number of occupationally exposed participants recruited and the number of confirmed MERS-CoV infections among these participants, and/or the number of participants with serological evidence of MERS-CoV infection. It should also include the number of dromedary camels tested and the proportion with laboratory evidence of MERS-CoV infection.

It is also important to fully document the study design, including recruitment methods, eligibility criteria, techniques for determining MERS-CoV infection and the outcome measurements, in order to assist the interpretation of the findings.

An integrated approach which engages both researchers and stakeholders should be used for conducting dissemination activities in joint efforts by the researchers involved and advisory committee members.

Dissemination activities could include:

- Submitting progress and final research reports to national Ministries of Health and Agriculture and to WHO.
- Publishing the research findings in peer-reviewed journals and making them available in open access format.
- Organizing meetings/seminars/workshops involving a panel of the research team beside other research experts (from human and animal health) to discuss the research findings and how they may influence public health interventions and policies.
- Developing policy briefs for national human and veterinary health authorities

COMMENT: The timely dissemination of the results of this study are critical in understanding transmission of the MERS-CoV virus to inform guidance for policy to direct national and international public health responses.

ACKNOWLEDGEMENTS

This generic protocol was adapted from a protocol developed by the Consortium for the Standardization for Influenza Seroepidemiology (CONSISE), a global partnership aiming to develop influenza investigation protocols and standardize seroepidemiology to inform public health policy for pandemic, zoonotic and seasonal influenza. This international partnership was created out of a need, identified during the 2009 H1N1 pandemic, for better (standardized, validated) seroepidemiological data to estimate infection attack rates and severity of the pandemic virus and to inform policy decisions. More information on the CONSISE network can be found on their website: www.CONSISE.tghn.org.

Other investigation tools for MERS-CoV can be found at: http://www.who.int/emergencies/mers-cov/en/

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We would also like to acknowledge the Supreme Council of Health in Qatar for sharing their MERS-CoV seroepidemiology questionnaires used in a similar study conducted in 2014 (Appendix B).

REFERENCES

1. WHO. World Health Organization. Emergencies. MERS CoV. Available at: http://www.who.int/emergencies/mers-cov/en/Last accessed 26 October 2017. 2012-2017.

2. The WHO MERS-CoV Research Group. (2013) State of Knowledge and Data Gaps of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in Humans. *PLoS Currents Outbreaks*. Published, 2013 Nov 12. Edition 1. doi: 10.1371/currents.outbreaks.0bf719e352e7478f8ad85fa30127ddb8.

3. Azhar, E. I., El-Kafrawy, S. A., Farraj, S. A., Hassan, A. M., Al-Saeed, M. S., Hashem, A. M., & Madani, T. A. (2014). Evidence for camel-to-human transmission of MERS coronavirus. *New Engl J Med 370* (26): 2499-2505.

4. Haagmans BL, Al Dhahiry SHS, Reusken CBEM, Raj VS, Galiano M, Myers R, et al. (2014) Middle East respiratory syndrome coronavirus in dromedary camels: an outbreak investigation. *Lancet Infect Dis*. 14(2): 140-5.

5. Park, S., Wernery, U., Corman, V. M., Wong, E., Tsang, A., Muth, D....Drosten, C. (2015). Acute Middle East Respiratory Syndrome Coronavirus Infection in Livestock Dromedaries, Dubai, 2014. *Emerg Infect Dis*, *21*(6), 1019-1022.

6. Perera RA, Wang P, Gomaa MR, El-Shesheny R, Kandeil A, Bagato O, et al. (2013) Seroepidemiology for MERS coronavirus using microneutralisation and pseudoparticle virus neutralisation assays reveal a high prevalence of antibody in dromedary camels in Egypt, June 2013. *Euro Surveill* 18(36): pii=20574.

7. Reusken CB, Haagmans BL, Müller MA, Gutierrez C, Godeke GJ, Meyer B, et al. (2013) Middle East respiratory syndrome coronavirus neutralising serum antibodies in dromedary camels: a comparative serological study. *Lancet Infect Dis*. pii: S1473-3099(13)70164-6.

8. Meyer B MM, Corman VM, Reusken CBEM, Ritz D, Godeke G-D, et al. (2014). Antibodies against MERS coronavirus in dromedary camels, United Arab Emirates, 2003 and 2013. *Emerg Infect Dis* [Internet] 2014 Apr [ahead of print]. 2014; http://dx.doi.org/10.3201/eid2004.131746.

9. Saqib M, Sieberg A, Hussain MH, Mansoor MK, Zohaib A, Lattwein E, Corman VM (2017). Serologic Evidence for MERS-CoV Infection in Dromedary Camels, Punjab, Pakistan, 2012–2015. *Emerg Infect Dis*, 23(3), 550–551. http://doi.org/10.3201/eid2303.161285

10. Miguel E, Chevalier V, Ayelet G, Ben Bencheikh MN, Boussini H, Chu DK, Peiris M. (2017). Risk factors for MERS coronavirus infection in dromedary camels in Burkina Faso, Ethiopia, and Morocco, 2015. *Euro Surveill* 22(13), 30498. http://doi.org/10.2807/1560-7917.ES.2017.22.13.30498

11. Corman VM, Jores J, Meyer B, Younan M, Liljander A, Said MY, Müller MA (2014). Antibodies against MERS Coronavirus in Dromedary Camels, Kenya, 1992–2013. *Emerg Infect Dis*, 20(8), 1319–1322.

12. Guery B, Poissy J, el Mansouf L, Séjourné C, Ettahar N, Lemaire X, et al. (2013) Clinical features and viral diagnosis of two cases of infection with Middle East Respiratory Syndrome coronavirus: a report of nosocomial transmission. *Lancet.* 381(9885): 2265-72.

13. Hijawi B, Abdallat M, Sayaydeh A, Alqasrawi S, Haddadin A, Jaarour N, et al (2013). Novel coronavirus infections in Jordan, April 2012: epidemiological findings from a retrospective investigation. *East Mediterr Health J* 19(Supplement 1): S12-S8.

14. Memish ZA, Zumla AI, Al-Hakeem RF, Al-Rabeeah AA, Stephens GM (2013). Family Cluster of Middle East Respiratory Syndrome Coronavirus Infections. *N Engl J Med* doi: 10.1056/NEJMoa1303729.

15. Pebody RG, Chand MA, Thomas HL, Green HK, Boddington NL, Carvalho C, et al. (2012) The United Kingdom public health response to an imported laboratory confirmed case of a novel coronavirus in September 2012. *Euro Surveill.* 17(40): pii=20292.

16. Corman VM, Müller MA, Costabel U, Timm J, Binger T, et al. (2012) Assays for laboratory confirmation of novel human coronavirus (hCoV-EMC) infections. *Euro Surveill* 17(49): pii=20334.

17. Buchholz U, Müller MA, Nitsche A, Sanewski A, Wevering N, et al. (2013) Contact investigation of a case of human novel coronavirus infection treated in a German hospital, October-November 2012. *Euro Surveill* 18(8): pii=20334.

18. Perera, R. A., Wang, P., Gomaa, M. R., El-Shesheny, R., Kandeil, A., Bagato, O., ... & Li, M. (2013). Seroepidemiology for MERS coronavirus using microneutralisation and pseudoparticle virus neutralisation assays reveal a high prevalence of antibody in dromedary camels in Egypt, June 2013. *Euro Surveill* 18(36): 20574.

APPENDICES

APPENDIX A: QUESTIONNAIRES FOR OCCUPATIONALLY EXPOSED PARTICIPANTS

The following questionnaires have been developed for this study:

- High risk group questionnaire: Abattoir worker
- High risk group questionnaire: Camel farm/ barn/ ranch worker
- High risk group questionnaire: Animal market worker
- High risk group questionnaire: Quarantine worker

APPENDIX B: POPULATION SPECIFIC QUESTIONNAIRES USED IN QATAR

(Provided separately)