

MERS Coronavirus at the Human - Animal Interface

Elmoubasher Farag

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MERS Coronavirus at the Human-Animal Interface

MERS Coronavirus op de mens-dier interface

Thesis

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By

Elmoubasher Abubaker Abd Farag
borne in New Halfa, Sudan

Doctoral Committee:

Promotor: Prof. dr. M.P.G. Koopmans

Other members: Prof. dr. M.D. de Jong
Dr. J.L. Nouwen
Prof. dr. J.A. Stegeman

Copromotors: Dr. C.B.E.M. Reusken
Dr. B.L. Haagmans



أَفَلَا يَنْظُرُونَ إِلَى الْإِبِلِ كَيْفَ خُلِقَتْ ﴿١٧﴾

“Then do they not look at the camels - how they are created?”

Verse (88:17) of chapter (88) sūrat l-ghāshiyah (The Overwhelming): Quran.

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Chapter 1

General introduction



INTRODUCTION TO EMERGING INFECTIOUS DISEASES

Infectious diseases are a continuing threat to all persons, regardless of age, sex, lifestyle, ethnic background, and socioeconomic status [1, 2]. The World Health Organization (WHO) defines Emerging Infectious Diseases (EID) as “diseases of infectious origin whose incidence in humans has increased within the recent past or threatens to increase in the near future”. These also include those infections that appear in new geographic areas or increase abruptly. The new infectious diseases and those which are re-emerging after a period of quiescence are also grouped under emerging infectious diseases” [3]. Three conditions are considered to identify a disease as emerging infectious disease: (a) they affect human beings for the first time, (b) they have happened in the past, involving only few persons in remote areas, however, have recently gained new epidemiological features, or (c) they have happened along the course of mankind history, but have only recently been identified as distinct diseases due to a contagious organism [1]. There are two main groups of emerging pathogens: newly emerging and reemerging infectious diseases [4]. According to the definitions and categorization used in literature, newly EIDs include new, previously indeterminate diseases (combining categories a and c), whereas reemerging infectious diseases are old diseases with new features (category b). By definition, these features would involve new geographical territories and/or populations, and different epidemiological and clinical attributes [5].

Historical background

Historically, it is believed that communicable diseases have been emerging and reemerging over millennia. While emergence of diseases can vary from country-to-country, location-to-location or population-to-population, their occurrence in populations has significantly increased within the recent past or the near future. Nevertheless, EID comprise a substantial proportion of the most lethal pandemics in human history, including the smallpox epidemic of 1520-1521, and the epidemics of measles [5].

Recently in the twenty-first century, SARS was reported to be the first severe EID. This followed from the new civet cats’ coronavirus in Guandong province, China in 2003. In March 2009, H1N1 influenza was reported in Mexico, USA, followed by spread to the rest of the world [6]. The detection of the MERS-CoV in human Saudi Arabia during 2012 was the first reported novel epidemic in the Arabian Peninsula, an event that has affected up-to-date 27 countries worldwide.

The most silent modern example of an EID is HIV/AIDS, which is thought to have emerged a century ago, and caused 35 million deaths so far [4]. From 1940 to 2004, 335 EID outbreaks have likely emerged worldwide [11], and over 30 new infectious agents were reported globally over the last thirty years [5]. Recently, H5N1 and H7N9 avian influenza, the pandemic H1N1 influenza A, SARS, Ebola, MERS, West Nile fever, measles, Nipah virus, dengue, and Zika have got much international focus for the significant morbidity and mortality in some [5,6,8,9]. Despite these examples, infectious diseases were no longer

considered as the major cause of death in the industrialized countries since the middle of the 20th century, [5].

Burden and cost

It is generally accepted that EID account for 26% of annual deaths worldwide [10]. 13% (177/1,407) of the accounted human pathogens are regarded emerging or re-emerging. Of these, 37% are viruses and prions and 25% protozoa [5]. Of the 335 observable EID events, over 60% were considered zoonotic [11] and more than 75% of these EID have arisen from the wildlife [5]. Epidemics or pandemics resulting from these emerging and re-emerging agents typically cause high mortality and morbidity rates. Their potential to spread fast over large geographical areas is a regular cause for panic. In less than 9 months, SARS infected 8,439 persons, 812 of them died. Pandemic influenza A (H1N1) 2009 virus – although considered to be relatively mild- resulted in 17,000 deaths; of which, 12,000 were in the United States of America alone [6]. MERS-CoV caused 2399 cases and 827 deaths worldwide since March, 2019 when it was first detected in 2012 [12]. Apart from the health impact, EID also may lead to severe economic consequences exemplified by reduced tourism, business and export; reduced developmental and security challenge. Should an epidemic of avian influenza hits Southeast Asia, the costs might mount to US\$283 billion, according to the estimations of the world bank [13]. SARS was associated with lost economic activity, estimated to cost around \$40 billion [14]. The 2014 epidemic of Ebola resulted in 28,639 reported cases along with 11,316 deaths in West Africa, and the 2015 epidemic has estimated to cost Guinea, Liberia, and Sierra Leone \$2.2 billion in GDP [15].

Dynamics of emerging infectious diseases

For a variety of reasons, it is obvious that novel pathogens have the potential to keep emerging and spread across the globe, straining public health authorities. Emergence and re-emergence of a given pathogen is a consequence of host-pathogen interactions that change with alterations in a range of environmental factors [7]. This includes adaptation of pathogens originating in animals, rendering them transmissible to human beings [5], but many more factors can change disease dynamics [2, 4, 16]. These include demographic factors, international travel, socioeconomic factors, environmental factors, animal and human health, man-made ecological changes, global warming and inadequate public health infrastructure [3]. With special reference to zoonosis, Liu et al. [9] considered seven determinants affecting emergence and reemergence; livestock production, pathogen mutation, population growth, food safety, urbanization, climate change, and deforestation.

Public health interventions to prevent EIDs

While the availability of reliable epidemiological data are important to launch an effective prevention and control measures to combat EID, fostering public health strategies that ensure elimination of a organisms from its reservoir or blocking its route of infection is essential. These interventions should include food safety, sewage treatment and disposal,

safe water, control of animal movement, as well as vaccination programs [1]. A rapid response mechanism [5], availability of strategic surveillance plan, strengthening of the laboratory networking, research partnership and information sharing [5,7], a prepared regulatory framework, effective reporting system, health education [9] are important for controlling and preventing EID [1].

As many EID are zoonoses, the control of zoonotic diseases in the animal reservoir and prevention of transmission to humans can be useful as well as cost-effective to both human and animal populations. However, particular interventions, like culling, are hard to implement without collaboration from farmers' who, in turn, may not cooperate if the compensation granted to them was perceived to be insufficient, as experienced in the H5N1 avian influenza outbreak back in 2006 [13]. The choice of an effective means of communication, education and advocacy is imperative to ensure that public health messages reach stakeholders. Long-term socio-economic negative consequences can occur if such strategies were not followed. The reaction to bovine spongiform encephalopathy (BSE) in England in the 1990s sets a good example for the potential to lasting economic losses [7].

Middle East Respiratory Syndrome

Coronaviruses (CoVs) are group of viruses under the *Coronaviridae* family [17] that can infect a wide variety of hosts, including birds, domestic and wild animals and humans [18, 19]. It is estimated that about 30% of common cold cases in the human population are caused by CoVs.

Since the first detection of a Middle East Respiratory Syndrome coronavirus (MERS-CoV) case in 2012 [20], studies were done on the possible source of infection. As a result, dromedary camels were identified as the main source of human infection of MERS-CoV [21-23]. Like Ebola in West Africa [24], MERS-CoV, which until now occurs sporadically across the Middle East, could transmit among humans and under certain conditions could potentially cause harm to many people. Some large outbreaks of community and healthcare associated infection have been reported, but all of them are secondary or tertiary in nature and mostly in healthcare settings [25]. It has been found that the Arabian Peninsula is the hot spot of MERS index cases [26], and that MERS-CoV antibody is shown to be common in persons with close camel contact [27]. However, there are many indices of MERS-CoV cases in human, where the route of infection is still unknown [28, 29]. In view of the above, evidence-based prevention and control of MERS-CoV needs knowledge of the human-animal interface.

History of MERS-CoV infection

A novel coronavirus was isolated from a Saudi man, who was suffering from severe acute pneumonia and died in June 2012 [20]. Subsequently, another Qatari man got severe pneumonia. Coronavirus isolated from the Qatari man was similar to the isolate reported the first case. On 30 November 2012, WHO has reported a similar case in Jordan [30,31].

By 2013, additional cases were confirmed from UAE, Oman, Kuwait, Italy, Tunisia, France, Spain, and UK. It was found that the origin of the disease was Arabian Peninsula and the reports in Europe and Africa were linked to Arabian Peninsula by travel. In 2013, the Coronavirus Study Group of the International Committee on Taxonomy of Viruses (ICTV) has agreed to give the new virus the name: Middle East respiratory syndrome coronavirus (MERS-CoV) [32].

Initial evidence on the possible association with animals came from the detection of anti-MERS CoV antibodies in dromedary camels' sera, urine and milk [33]. In 2014 MERS-CoV RNA was identified in nasal swabs from camels owned by a Qatari man (first case of MERS in Qatar) who was suffering from severe acute respiratory syndrome. The viral genome from samples collected from the camels and the Qatari man was similar [34]. Subsequently, MERS-CoV antibodies were reported from camels in the Kingdom of Saudi Arabia (KSA) [35,36], Egypt [37], and Kenya [38]. By 2017, MERS-CoV has been found widespread distribution in dromedary world throughout the Arabian Peninsula, Africa, South Asia, and Canary island of Spain [39].

MERS in Humans

Six known human coronaviruses (HCoV) have been identified so far. These are divided into α -coronaviruses, represented by HCoV-229E and HCoV-NL63, and β -coronaviruses represented by HCoV-HKU1, HCoV-OC43, SARS-CoV and MERS-CoV [17]. In humans, MERS-CoV infection may be asymptomatic, or symptomatic causing signs ranging from mild complaints to severe acute respiratory syndrome [40-44]. The incubation period varies from 2-14 days [45]. Symptomatic illness may include cough, shortness of breath, fever, sore throat, headache, hemoptysis, nausea, vomiting, diarrhea, and serious complications might ensue leading to multi organ failure and death [42-44].

WHO was notified of 2428 confirmed human cases that caused 838 deaths in 27 countries in the world- as of 5th of June, 2019 [25]. Asia, Europe, Africa, and North America all reported MERS cases. Till July 21, 2017, the highest prevalence of cases has been reported from the Middle East, mainly from the KSA where 1672 were confirmed cases. The Republic of Korea ranks second highest scoring 185 cases due to a large healthcare associated outbreak following a case imported from KSA [46]. A national serosurvey was conducted in KSA to determine the seroprevalence of MERS-CoV antibodies among the general population, and in particular those in contact with camels found an overall prevalence of 0.15% (15/10009). Antibodies were more prevalent in men (0.25) than women (0.05%), and higher in camel workers (2.3%) and abattoir workers (3.6%)[47]. In another study in hospitalized patients in KSA, seroprevalence of MERS CoV in suspected patients was 0.7% (384/57363). In Kenya, 2 persons out of 1122, not directly linked to camels but living in an area where camels are widespread, were seropositive for MERS-CoV antibodies [48].

MERS in camels

To date, three types of coronaviruses were identified in camels; human OC43-related camel coronavirus, human 229E-related camel alpha-CoV, and MERS-CoV HKU23 [49, 50]. There is also camel coronavirus, UAE-KKU23 that is under β -coronavirus 1 [51]. It was hypothesized that camels are the intermediate animal host that allowed the ancestral MERS-CoV in bats to cross the species barrier to enter humans [49]. In camel, MERS-CoV causes asymptomatic to mild respiratory tract disease. If symptoms appear, mucopurulent lacrimal and nasal discharges are common [52-54]. The nasal passage, trachea, bronchioles can be involved with mild inflammation but pneumonia has not been observed [52]. More than 70% of the dromedary camels tested worldwide are positive for MERS-CoV antibody [35]. Based on surveillance that relied on the detection of MERS-CoV antibody or the RNA, Asian and African countries in addition to Canary Islands of Spain have all reported MERS-CoV syndrome in camel population. MERS-CoV antibody and/or nucleic acid has been detected in camels of KSA [35,54], Qatar [34,55], UAE [56], Jordan [57], Oman [58], Iraq [59], Iran, Pakistan [60], Sudan, Somalia, Egypt [37,61], Nigeria, Tunisia [62,63], Burkina faso, Morocco and Ethiopia [62,64], Kenya [38] , Mali [65] and Canary Islands of Spain [33,66]. Given that only MERS-CoV antibodies and not the virus was detected in camels in Chad, Libya, Mali, Sudan and Ethiopia, there is a possibility that the disease is enzootic in these countries [37,63]. Seroprevalence of MERS-CoV antibody is higher in adult than young camels [56,57,60,64,67-69]. However, while the dromedary camels of Kazakhstan are livestock camels they were found negative for MERS-CoV prevalence [70]. This may be due to lack of contact with camels of the Arabian Peninsula or Africa. Conversely, young camels observed more often shed virus than adults [35,64,69,71,72]. They are considered to facilitate virus amplification in dromedary camel populations [38,64].

The prevalence of MERS-CoV in camels is also related to their management. Restriction of movement can reduce MERS-CoV transmission in camel herds [35,65]. Transmission of the virus is believed to be density dependent, as higher seropositivity rate is proportionate to the herds' size. [64]. Studies showed that imported camels are more seropositive with higher rate of PCR detection compared to local camels in Egypt [61] suggesting that camel movement and trade constitute a key risk factor to transboundary transmission of MERS-CoV to low prevalence countries. Since winter is the season of calving, and that higher rates of MERS-CoV infections among young camels is documented [56,57,60,64,67-69], chances for the virus transmission are higher when large numbers of young camels which shed the virus moved [37,54]. Large quantity of viral shedding through nasal secretions. Therefore, the virus can spread via direct contact between camels and to humans. Additionally, transmission can occur through fomite, milk, and feces or even by the large nasal droplets [52, 35, 69].

MERS in other animals

MERS-CoV nucleic acid was detected from one Egyptian tomb bat in KSA [73]. MERS-CoV replicated efficiently in Jamaican fruit bats (*Artibeus jamaicensis*). Despite no clinical signs were seen among the infected bats, yet they shed virus from its intestinal tract as well as its respiratory system for up to 9 days [74]. In China, studies on *Vespertilio superans* bats Lineage C betacoronavirus was identified [75]. Natural infection of MERS-CoV was detected in alpaca [76]. Upon experimental infection, alpaca were found to shed virus through oral and nasal routes [77]. Domestic pigs replicate low levels of the virus and may shed it [78]. Asymptomatic MERS-CoV infection along with viral shedding has been detected in rabbits [79]. No Bactrian camel was yet found positive to MERS-CoV infection either by antibody or RNA detection [70,80,81]. Other domestic animals like cattle, goat, buffalo, horse, and donkey were considered refractive to MERS-CoV infection [33,35,56,61,69,82-85].

Viral shedding

Viral shedding from humans

MERS-CoV is shed through respiratory secretions, urine, and stool [86,87]. Tracheal aspirates, sputum, nasal and throat swabs, bronchoalveolar fluids were used by researchers to diagnose MERS cases [42,43,86,88] indicating shedding of the virus through respiratory system. Several studies affirmed that samples taken from the lower respiratory tract yield higher viral loads compared to samples taken from the upper respiratory tract [86,89]. Viral load or nucleic acid concentration was higher in respiratory samples than urine or stool samples. In urine or stool samples, the viral RNA concentration was close to the lowest detection limit of the assay [86].

After symptoms onset, MERS-CoV continued to be detected in samples collected from respiratory specimens till day 25. [88]. Investigating the viral load and shedding duration from day 37 of MERS-CoV patients, Corman et al. [87] could detect the virus throughout the duration of the investigation. Viral RNA was detected from 14.6% of the stool samples up to day 23, and from 2.4% of the urine samples up to day 5 [89]. He also concluded that the intensity as well as the timing of the respiratory viral shedding in patients with MERS is similar to that of patients with severe acute respiratory syndrome (SARS). He attributed this to insufficiency of the resulting neutralizing antibodies to clear the infection.

Various environmental surfaces, in particular those that are frequently touched, were found to play a role in MERS-CoV transmission: bed sheets, patient rooms, bedrails, IV fluid hangers, anterooms, air-ventilating equipment, x-ray devices, and medical devices were found to be contaminated by MERS-CoV [88,90]. The identified secondary transmission rate among house hold contacts of MERS-CoV patients were only 5% [86] and blood samples seems to contain no virus particles [86,89].

Viral shedding from camel

Investigating MERS-CoV replication and viral shedding pattern in camels, it was found that the respiratory, digestive and reproductive systems of camels can support MERS-CoV replication throughout the silent course of infection [35,58,61,91]. This has been evidenced by retrieval of viral nucleic acids from nasal, conjunctival nasopharyngeal and rectal swabs as well as milk samples from apparently healthy camels. Additionally, the virus was isolated from samples collected from nasal secretions and faeces [50,55,92]. Airborne infection was evidenced also via the detection of viral RNA from air samples collected from a barn owned by an infected patient [93]. Viral antibodies were also demonstrated in milk samples of camels [21]. Viral loads were found to be higher in nasal samples, but less shedding was detected in oral and rectal samples [91]. Small, yet not infective, quantities of viral nucleic RNA particles were detected in exhaled breath [53].

Evidence of camel to human of transmission MERS-CoV

A wide body of research documented the zoonotic nature of MERS-CoV [94]. The sequenced genomes of camel MERS-CoV were similar to those of human MERS-CoV [34,36,95,96]. Serological studies showed that MERS-CoV was circulating in camels before human infection was recognized [22]. The successive investigations of Memish et al. [36] and Haagmans et al. [34] are supporting this assumption.

Summary and knowledge gaps

MERS-CoV was isolated from both young and adult camels. Thus, the risk of cross species MERS-CoV transfer from camel to human is higher from calves than from adult. Direct contact with infected camel, especially contact with camel excreta like nasal discharge, feces, milk, contaminated air (Figure 1) increase the risk of camel human infection. Kissing of camels is a tradition among Arabian people as they cheer their camels [44]. This tradition increases the risk of direct transmission of MERS-CoV.

Raw milk consumption directly after milking is another tradition that implies high risk of transmission, as raw milk can be a source of infection for consumers [84]. In comparison with camel farm workers, camel workers handling camels in quarantine at live animal markets and slaughterhouses have higher risk of infection, most likely due to intensity of contact and density of animals, but other factors like animal stress can not be ruled out. Given the epidemiology of MERS-CoV, it is likely that camel farms with low or no biosecurity get more MERS-CoV infection; subsequently the workers of these farms have increased chances to get MERS-CoV infection than those serve in farms characterized with strong biosecurity standards. An example is the risk for MERS-CoV incursion on camel farms with frequent import of camels from other farms or countries.

The possible epidemiological role of other species in perpetuation of MERS-CoV remain to be investigated. Observingly, increasing contacts between human and animals from one side and between animals and animals from the other side have been brought about

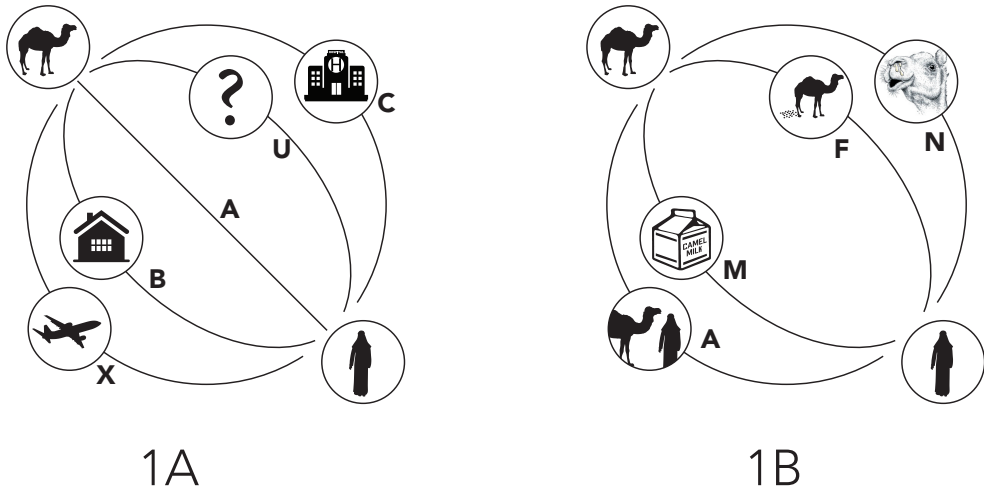


Figure 1: Speculated MERS-CoV infection sources. Figure (1A) shows routes of MERS-CoV transmission from Camel to Human; 'A' direct contact with camel, 'B' household, family or community contact, 'C' hospital or nosocomial contact, 'U' unknown source rather than A, B or C and 'X' through travel to Arabian Peninsula and infection by A, B, C or X route. Figure (1B) shows source of infection of MERS-CoV from camel to human 'N' infection through nasal discharge, 'F' feces, 'M' milk, and 'A' airborne infection.

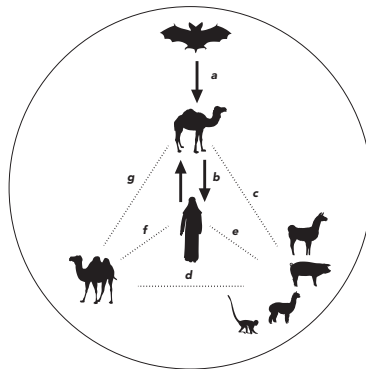


Figure 2: Anticipated future threat of MERS-CoV being a multi-species complex disease (pink round). This might be expected through the scenario that MERS-CoV have been speculated to derive from bat to dromedary camel (a). Human get the infection from dromedary camel (b). Alpaca, llama, pig and monkey have been found susceptible to MERS-CoV having the possibility of getting infection from human (c) and/or camels (d). There is chance for Bactrian camel to be susceptible to MERS-CoV and get infected from exposure to dromedary camel (g), alpaca, llama, pig and monkey (d) or human (f).

vastly for commercial and/or industrial purposes. Theoretically, among other factors, this might offer the virus an appropriate epidemiological chance to gain the capability to cross the species barrier (Figure 2). At this junction, an increase risk of the MERS-CoV might be established.

There are persistent critical gaps in what we know about MERS-CoV. Particularly, factors precipitating MERS-CoV emergence and transmission at the human-animal interface needed to be indentified and better elucidated. This research proposal aims at addressing some of these critical gaps and establishing better understanding of the potential role that dromedary camels and other animal sources play turning MERS-CoV into an emerging zoonotic disease. Since MERS-CoV is an emerging zoonotic infectious disease, many studies recommend to embrace intersectoral collaboration between health, veterinary, and environmental disciplines including the private stakeholders, and adopt a collaborative one-health approach to shoulder the responsibility of combating the epidemic [97].

THE OUTLINE OF THE THESIS

While camels are recognized as a natural host for MERS-CoV and a source for zoonotic introductions to humans, only a small percentage of the primary cases with documented direct contact with dromedary camels can be explained, leaving the door open to other possibilities. These possibilities include food-borne transmission and other zoonotic origins. The studies presented here were done as part of the public health preparedness and response activities in Qatar and aimed to address essential knowledge gaps important for public health.

Objectives and main question of the studies:

To understand MERS-CoV dynamics at the human-animal interface by identifying factors that potentiate the emergence, transmission and spread of MERS-CoV in Qatar.

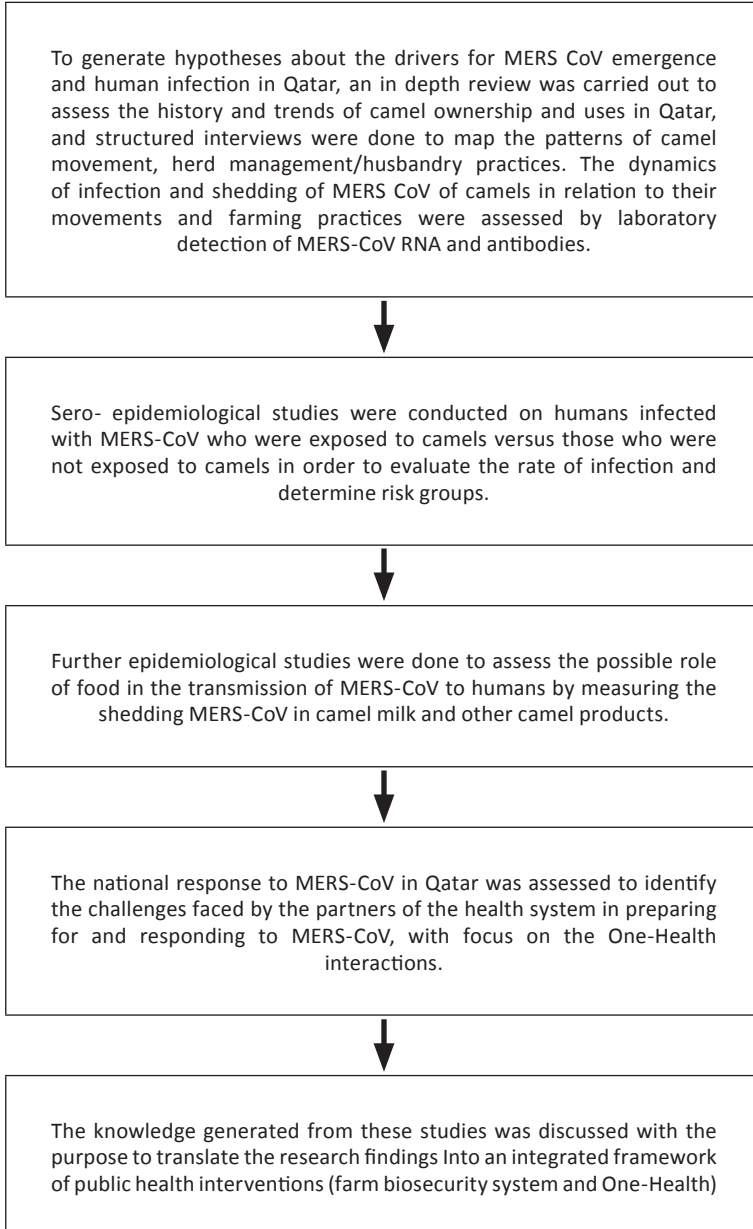
To explore the strengths and challenges faced by health system partners Qatar in preparing for and responding to MERS-CoV outbreak.

In order to address the above objectives, we raised the following questions:

1. What are the characteristics, risk factors for infection and outcome among the confirmed MERS-CoV cases in Qatar? (Chapter 2).
2. What is the evidence of MERS-CoV infection in humans exposed to camels? (Chapter 3 and Chapter 4)
3. What are patterns of shedding of MERS-CoV in camels in different situation and uses? (Chapter 5).
4. What are the challenges faced by health system partners in preparing for and responding to MERS-CoV outbreak? (Chapter 6).
5. How the One-Health approach was informative to surveillance and response to the emergence of MERS-CoV ? (Chapter 7).
6. What are the characteristics, risk factors and prevalence of MERS-CoV infection and outcome in camels? (Chaper 8)

7. What are the drivers of MERS-CoV emergence and spread at the Camel-human interface in Qatar and how does that influence risk of exposure of humans to MERS-CoV? (Chapters 9).

The objectives will be addressed through the following tasks:



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Chapter 2

Middle East respiratory syndrome
in Qatar: a retrospective study of
the laboratory confirmed cases
between 2012-2019

Manuscript in preparation

ABSTRACT

Middle East respiratory syndrome (MERS) is a human disease caused by a coronavirus (CoV). In the present study, we reviewed and investigated the laboratory confirmed MERS cases reported in Qatar between September 2012 and February 2019. Epidemiological, demographic, and clinical characteristics of MERS cases were obtained using a structured questionnaire and by reviewing the MERS-CoV surveillance reports at Ministry of public Health (MoPH), Doha, Qatar. A total of 24 individuals - all adults - were identified; 23 were male and only 1 was female. Eight patients died and the case-fatality rate rose with age. Most patients (n=14) had underlying medical disorders, including diabetes (n=7), hypertension (n=6) and chronic artery disease (n=5). The average days of hospitalization of the MERS patients was 21.5 days and after confirmation, virus shedding continued for 11-13 days. Different from the epidemiological patterns seen in KSA and Korea, the majority of cases (n=19) most likely resulted from direct or indirect camel exposure. The Qatar policy implemented in 2013 to test every hospitalized patient with camel contact regardless of the symptoms observed and the employment of a One Health team during routine MERS-CoV cases investigations in the field, may have significantly reduced the subsequent spread of MERS-CoV in Qatar. The lack of transboundary camel and human movement between Saudi Arabia and UAE with Qatar due to the 2017 blockade also may have limited the number of MERS cases in Qatar.

Key words: MERS-CoV, epidemiological, demographic, clinical characteristics, transmission dynamics, Qatar

INTRODUCTION

Middle East Respiratory Syndrome Coronavirus (MERS-CoV) was first detected in the Kingdom of Saudi Arabia (KSA) in 2012 [1]. Subsequently, human cases were noted in Qatar and Jordan with similar clinical features [2, 3] and the first case of human to human MERS-CoV transmission was reported from the United Kingdom in 2013 [4]. Between September 2012 and April, 2019, a total of 2428 cases and 838 deaths have been reported in 27 countries across the world [5]. All cases reported outside the Gulf States had a travel history and/or residence in one of the Arabian Peninsula countries; Saudi Arabia, United Arab Emirates (UAE) or Qatar [6]. The disease incubation period ranges from two days to two weeks [7] and the clinical manifestations represent a wide spectrum of disease ranging from mild to severe respiratory syndrome, influenza-like illness with mainly lower respiratory tract symptoms, complicated by pneumonia, acute respiratory distress syndrome, and organ failure [7-10]. Asymptomatic MERS cases range from 0% to 28.6% [11].

MERS-CoV has been identified as a both community [12, 13] and hospital-acquired infection [14, 15]. Elderly persons and those with multiple comorbidities were found to be at a higher risk of acquiring the infection, developing complications and may succumb to the infection [16]. The epidemic focus of MERS in the Arabian Peninsula has been attributed to spill-over from the widespread population of dromedary camels [17] with amplification during hospital outbreaks in KSA, Korea, Jordan and UAE [15]. High viral loads are typically detected in nasal fluids of infected dromedaries [18] suggesting direct contact can be a source of infection from camel to camel [18] and camel to human [19]. Several studies suggested that the camel-breeding season, which occurs during winter, plays an important role in MERS-CoV spread, as young camels are typically found to shed the highest loads of MERS-CoV [17, 18, 20, 21]. It remains to be seen, which other factors influence transmission and subsequently play a role in the epidemiology of MERS.

Unlike the documented epidemiological patterns seen in KSA where MERS-CoV was reported to have spread in hospitals or household settings, in Qatar the pattern observed seems to be sporadic in nature. Several factors were suggested to have driven MERS-CoV emergence in Qatar including the economic boom that paved the road for flourishing camel-related sports and business, enlarged population density with growing number of expatriates and the transformation of Qatari communities from Bedouin to urban sedentary lifestyle with rising records of co-morbidities like Type 2 diabetes, hypertension, dyslipidemia, obesity and other chronic illnesses. It is believed that the increasing number of camels and the ban of open grazing owing to the exacerbating impact of desertification were key to the virus spillover from camels to humans as large number of camels were placed in compact barns in which their care givers also live [22, 23].

This is the first paper to provide a descriptive review for all laboratory-confirmed MERS cases reported in Qatar 2012-2019 in attempt to contribute insight on the understanding of the human MERS-CoV transmission, epidemiology and the potential risk factors in Qatar

[22, 23]. The extrapolation of Qatar situation can be helpful for regional and global public health and veterinary scholars to foster a collaborative One-Health approach and hospital settings to manage emerging infections [22].

MATERIALS AND METHODS

Data collection

The data of all laboratory confirmed cases of MERS, reported between September 2012 and February 2019, that were investigated by the MERS-CoV One Health Investigation team [45] at the Ministry of Public Health (MoPH), Doha, Qatar were included in the present study.

Patient clinical data included co-morbidities, duration of clinical sign(s), date of hospitalization, disease outcome, and date of discharge/died. Patients epidemiological data such as patient's demographic characteristics, age, sex, occupation, travel history to Saudi Arabia or other countries in the Middle East, history of camel contact, nature and place(s) of camel contact, contact history (and nature) with possible other MERS patients, and camel related information such as area and type of the farm, farm biosecurity, camel breeding season, camel movement, camel health status and other risk factors related to camels were obtained by a structured questionnaire and field investigation.

Ethical approval:

As this research was done as part of the outbreak investigation efforts, ethical approval was waived from the Health Research Governance Department

Confirmation of MERS-CoV cases

All the clinical samples were screened using the Fast Track diagnostics real-time reverse-transcription polymerase chain reaction (rRT-PCR) assay, targeting the upE and ORF1a genes, respectively, as previously described [25]. A case was considered confirmed when both targets were detected according to the WHO guideline [26, 27] at the Influenza laboratory in the National Influenza Centre of Hamad Medical Corporation, Qatar.

Definitions

Confirmed case: A person with laboratory confirmation of MERS-CoV infection irrespective of clinical signs and symptoms [28].

Primary case: cases with laboratory confirmation of MERS-CoV infection with no direct epidemiological link to a human MERS case [19].

Secondary case: cases with laboratory confirmation of MERS-CoV infection, and with a direct epidemiological link to a human MERS case [19].

Unclassified case: cases with insufficient information, based on potential prior exposures to allow classification as a primary or non-primary case [19].

Direct camel contact: any physical contact (e.g., touching, feeding, cleaning, slaughtering, milking, assisting with birth, or other activities involving physical contact with dromedaries) with camels in the 14 days before symptom onset or when laboratory confirmation was reported [19].

Indirect camel contact: indirect exposure to dromedaries such as visiting camel areas (e.g., markets, racing tracks, farms) without directly touching a camel, or consumption of dromedary products (e.g., raw/unpasteurized dromedary milk, raw or undercooked dromedary meat, or other products derived from dromedaries, including urine) in the 14 days before symptom onset, or when laboratory confirmation was reported [19]. Moreover, cases who did not have direct camel contact but had contact with persons who had “direct camel contact” in the 14 days before symptom onset or when laboratory confirmation reported, was also considered as indirect camel contact.

No contact: any case that could not be defined as direct or indirect contact [19].

Travel history: travel history was considered if the patient traveled in any country of the Middle East in the 14 days before symptom onset, and laboratory confirmation was reported [26].

Risk factor and Comorbidity: any attribute, characteristic or exposure of an individual that increases the likelihood of developing a disease or injury was considered as risk factor [29]. Presence of additional diseases in relation to an index disease in one individual was considered as comorbidity [30]. Risk factors of MERS-CoV infection described previously, such as camel contact, increased age, different comorbidities were considered in this present study [10, 15, 16, 31-34]. Alcohol and smoking habit was also considered as risk factor.

Statistical analysis

All the data were inserted on Microsoft excel worksheets and the descriptive analysis, and frequencies were calculated using SPSS statistical program (v22.0, SPSS, Chicago, IL).

RESULTS

Demographic and clinical characteristics of the study population

A total of 24 cases and 8 associated deaths were reported during September 2012 to February 2019 in MoPH, Qatar. The highest numbers of positive cases (n=7) were detected in 2013. The majority of cases were male (n=23), with a mean age of 49 years (ranges from 22 to 74). The case fatality rates were higher (n=7) for persons >45 years of age and for Qatari (n=5) versus non-Qatari patients (n=3).

The majority of patients (n=19) were primary cases. Among the three secondary cases, case no 3 probably got the infection from Madina (KSA) [35]. Case no 7 (camel worker) was considered a secondary case as this patient had direct epidemiological link with case

no 6 (camel owner) [36]. However, three camels linked with patient no 6 and 7 were found positive for MERS-CoV [37]. Consequently, case no 7 can be considered as a primary case too. Case no 23 got the infection from case no 22 while sharing the living room. Seven cases traveled to KSA within two weeks before showing sickness. Three patients (Case no 7, 17 and 23) were detected by the One Health team during routine MERS-CoV case investigations; details were presented previously [24]. Two patients (Case no 7 and 23) were found MERS-CoV positive through contact tracing at the field level. Case no 23 was hospitalized for other health problems such as hypertension, diabetes, other respiratory disorders and later confirmed MERS-CoV positive. The Qatar One Health investigation team policy is to test MERS-CoV infection for every hospitalized patient with direct camel contact, regardless of the MERS symptoms. Case no 7 and 17 were asymptomatic at the time of hospital admission.

Figure 1 shows the course of infection of all patients. Upon testing suspected cases, the Qatar policy is to admit them to the hospital for isolation and to discharge when two consecutive screening assays (upE and ORF1 by rTR-PCR) are found negative for the virus. Hospitalization lasted on average 21.5 days. The patients had symptoms for less than a week before diagnosis and hospital admission. The most common observed symptoms were fever (n=21) followed by cough (n=18) and shortness of breath (SOB) (n=9) (Table 1). A total of 14 patients were identified with co-morbidities (Table 1). The most common co-morbidity factors of infected patients were diabetes mellitus (n=7), followed by hypertension (n=6), and coronary artery disease (n=5). In addition, associated risk factors were smoking (n=8) and alcohol use (n=5). Out of 14 patients with comorbidities, 5 died within 17 days of hospitalization. The others (n=9) were discharged on average 36 days after hospitalization.

Of the 10 patients without comorbidity and associated risk factors, 8 were discharged after on average 26 days hospitalization. The majority of the patients (n=20) started to shed virus at around 5 days upon start of symptoms and shed viral RNA for at least 2 weeks or more. Five patients died while still shedding viral RNA but three patients died after seemingly clearance of the infection. The median duration of shedding was 11 to 13 days.

MERS-CoV Epidemiology

The MERS cases in Qatar were reported with residency and putative exposure from Al-Shahanyia (13 from camel racing area, 2 from Al-Shahanyia camel market), followed by 7 cases from Doha (2 from Doha camel market, other 5 were living in different parts of Doha) and 1 case of each was from Abu Nakhla and Dukhan (Figure 2).

Cases were not evenly distributed over the year. In Figure 3, we show the number of cases in relation to camel activities as described previously [22]. We found that 12 cases were reported during August-October, coinciding with the weaning and training of young racing camels, whereas 7 cases occurred between March-June, which is the resting season for camels after the return from international races. Most of the patients had a history of

Table 1: Epidemiological characteristics of MERS-CoV cases and outcome in Qatar between September 2012 and December 2017.

Variable/Characteristics	MERS-CoV Positive n	Outcome (Expired/ Recovered)
Occupation		
<i>Camel related occupation</i>		
Camel owner	6	2/4
Camel worker	8	1/7
<i>Others occupation</i>		
Construction worker	1 ^β	0/1
Courier service employee	1 ^γ	1/0
Driver	1 ^β	0/1
Fish market worker	1 ^β	1/0
Geologist	1 ^γ	0/1
House wife	1 ^γ	1/0
Retired person	2 ^δ	1/1
Trader	1 ^ε	1/0
Unemployed	1 ^α	0/1
Symptoms		
Fever	21	7/14
Cough	18	6/12
Shortness of breath (SOB)	9	5/4
Fever and cough	17	5/12
Fever and cough and SOB	7	4/3
Others [§]	10	3/7
Comorbidity		
Comorbid	11	5/6
Diabetes mellitus	7	3/4
Hypertension	6	3/3
Coronary artery disease	5	3/2
Cardiovascular accident	2	2/0
Renal	2	1/1
Liver	1	1/0
Obesity	2	2/0
Asthma	2	2/0
Thyroid	1	0/1
Dyslipidemia	2	0/1
Others	1	0/1
Non-comorbid	13	3/10
Other risk factors		
Alcoholic	5	2/3
Smoker	8	4/4
Non alcoholic &/or smoker	16	4/12

§ Sore throat, body ache, back pain, dysuria, dyspnea, dizziness, abdominal pain, vomiting, diarrhea, urinary complaints, skin rash; α Used to visit camel farm frequently; β Living with camel farm or slaughter house worker; γ No direct or indirect contact with camel; δ Family had camel farm, family members had frequent contact with camels; ε Patient visited his family and drank camel milk within two weeks of symptoms developed

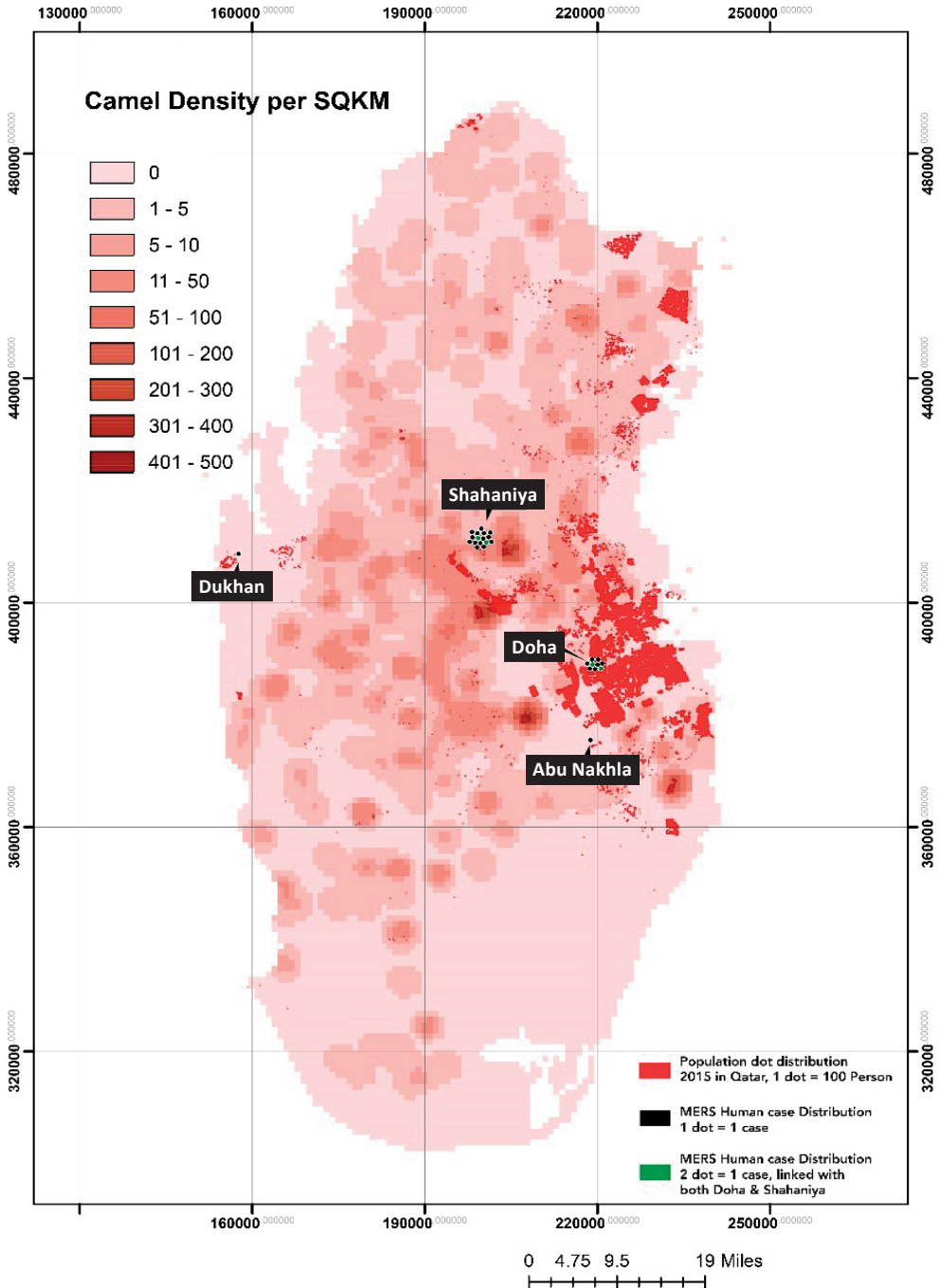


Figure 2: Spatial distribution of MERS-CoV cases in relation with camel and human population density distribution in Qatar.

MAJOR SEASONAL ACTIVITY OF CAMEL	LOCATION	SEASON											
		JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN
Weaning of the race camels	Breeding farms in whole Qatar	[Shaded area]											
Movement of the newly weaned camels	Breeding farms to Al-Shahaniya racing farms	[Shaded area]											
Camel Mating	Camel breeding farm throughout Qatar including Al-Shahaniya camel racing farms	[Shaded area]											
Camel race and show competition	Al-Shahaniya and movement of camels and workers throughout the region	[Shaded area]											
Resting season	Every camel farm in Qatar	[Shaded area]											
MERS-CoV patients		0	5	1	6	1	1	1	1	1	2	3	1

Figure 3: Relation between the reported cases and season of camel's activities in Qatar September 2012 to December 2017 (n = 24).

direct (n=16), followed by indirect (n=5) camel contact. The MERS-CoV patients had contact with 18 camel farms of which 16 were racing camel farms. None of the farms had any animal biosecurity system. All the 18 camel farms kept different species, including Qatari and non-Qatari camels, sheep, goats, and occasionally chicken, pigeons and dogs. Every farm had young camels less than one year, the owner's majlis (resting places) and worker's accommodation. Some of the camel farms had a history of hosting camels coming from other Gulf countries during the camel racing and show season. The majority of the camels had a history of movement outside the premises for racing practice, training, breeding, show competition and market. Some of the camels had history of international movement (mainly KSA and UAE).

DISCUSSION

Knowledge of disease epidemiology is essential to develop effective control strategies. Hitherto, several MERS-CoV epidemiological studies were conducted in KSA and other gulf countries [10, 15, 34, 38, 39] but not in Qatar. In the present study, we have been able to delineate 24 MERS cases in Qatar reported between September 2012 and February 2019. As shown in figure 1, 16 cases had a direct contact with camels, while 5 reported an indirect contact with camels. In agreement with previous studies [40-42], risk of MERS-CoV infection was linked to exposure to camels. Although, Qatari camel owners usually experience less frequent contact with their camels, the young non Qatari camel workers are involved in

feeding, milking, riding, training, cleaning farms, and providing health care to the sick camels, serving owners and their visitors. As these activities largely being performed with complete ignorance to the hygienic measures coupled with poor risk perception, farm biosecurity will be central to all prevention and control efforts [22, 42].

Consistent with previous reports [10, 38], CFR was higher among the elderly group (>45 years) in our population. Comorbidity patterns reflect the impact of MERS [41] and increasing age together with chronic morbidity were also emphasized in this study as risk factors. It was reported in KSA that more than 50% of cases aged 50 years old had diabetes [43], which was found compatible with our study. An additional explanation for the enhanced fatality rates in older patients could be the presence of senescence-associated immune vulnerability in these individuals and suboptimal immune reactivity following MERS-CoV infection. Further case-control studies are needed to define the effect of co-morbidities on susceptibility to and associated mortality from MERS-CoV infection.

Further, a high percentage of patients were male adults, which corroborates previous reports from different parts of the world [8, 14, 44]. However, there is no evidence that MERS-CoV has gender predisposition [45]. The observed gender-related rates could be simply due to the higher male exposure to camel population than females [45, 46]. Aside from the history of contact with camels or visiting a camel farm, our results did not point to a specific profession as a risk for MERS.

A seasonality pattern in the transmission of MERS-CoV in both human and camel has been reported, particularly during early months of winter (August-October) when young camels are weaned and involved in race training [17, 21, 47], as young camels are typically found to shed the highest loads of MERS-CoV RNA [18, 20].

However, such pattern is not obvious in Qatar as the MERS cases in Qatar were reported in different seasons, with the majority of cases in summer.

Unlike the documented epidemiological patterns seen in KSA and UAE [12, 14, 49] where MERS-CoV did spread in hospitals or household settings, Qatar cases were mainly primary and sporadic in nature. [48]. Only three secondary cases were reported in Qatar, of which one case was imported from KSA [35] and two from the community inside Qatar. The absence of hospital clusters may be due to the Qatar One Health approach to combat MERS-CoV [24] and the IP&C (infection protection and control) system in health care settings located at high risk zones in Qatar. The multidisciplinary One Health team including the community people helped to detect MERS patients early in the community [24], resulting in reduced secondary transmission of the virus from the primary cases. The IP&C settings may have helped to prevent nosocomial transmission of the virus in the hospital settings. The majority of cases (n=15) were reported from Al Sheehaniya area, where camel races take place. Since early epidemiological reports in 2013 suggested a link to camels in the MERS transmission, Al Sheehaniya Health Center implemented strict IP&C measures with a broader case definition for suspected MERS-CoV infection. According to this case definition, "Any person with an acute respiratory infection, with history of fever

and cough and indications of pulmonary parenchymal disease and any contact with a farm animal” will be considered a suspected case. This case definition is used in addition to WHO case definition for surveillance [28]. Any suspected case goes through strict triage, is admitted to isolation rooms where airborne IP&C standards are applied, and hospitalized till confirmed negative for MERS-CoV infection by rRT-PCR. The roles of every staff member at Al Sheehaniya health center to handle suspected and isolated MERS patients are clearly defined and strictly maintained.

Despite the WHO recommendation standard and droplet IP&C measure to handle cases suspected with MERS-CoV, the Qatar experience could be compared with the experiences of the other affected countries to provide recommendations. The Qatar experience highlights a significant guide to surveillance practice and investigation of suspected cases particularly in countries where camels are raised or when persons with chronic illnesses return from countries affected with the epidemic. While triaging cases with chronic diseases such as diabetes mellitus, coronary artery disease, renal diseases or hypertension, a history of contact with camels or visiting a camel farm should be a key question.

One MERS case was imported from KSA to Qatar [35]. The history of 7 patients travel to KSA might suggest a transboundary disease transmission in Qatar [40]. The MERS cases in Qatar were linked mostly either by Al Sheehaniya area (the main hub for camel race) or to the wholesale market which is the main center for camel trade. These two areas were major hubs for international camel gathering. After the blockade on Qatar started in May 2017 by KSA, UAE and some other Arab countries [50], camel trade and movement as well as movement of humans (camel workers and camel owners) between these countries stopped. No MERS cases were reported in Qatar from that time till now, whereas, MERS is continuously being reported in KSA, UAE, and some other Gulf countries [51]. These countries are interlinked by human and camel movement. Previous studies have shown that transboundary movement of humans (owners and workers) and camel could be a source of MERS-CoV transmission [22, 52]. Therefore, it is essential to study the origin and mode of transmission of MERS-CoV among the Gulf countries.

Another aspect that might have several implications on surveillance, case management, laboratory workload, and infection control and prevention is the virus shedding. While we are not sure about starting of the viral shedding in the present study, MERS-CoV shedding persists on average for 11-13 days, consistent with the findings of previous studies that have tested MERS-CoV genomic RNA [53-56]. On the basis of these results, IP&C precautions should be thoroughly applied for at least 1 month after symptom onset of patient infected with MERS-CoV.

There were several limitations in our study. First, patients were self-selected because of referral to hospital and being screened actively for MERS-CoV according to the seriousness of their clinical condition. Second, study is retrospective where reliance on clinical records was the only available choice of data and that has not been validated

or updated. Additionally, serology was not adopted as routine diagnostic measures for cases and contacts. Third, entry of case records was not done uniformly. The inconsistent approach in investigating the suspected cases is thought to have contributed to the lack of data related to the probable risk factors, based on the One-Health approach. These alterations caused some inconsistencies in the data acquired from the database that may limit the interpretation of the study results.

Nevertheless, this study confirms the importance of direct and indirect contact with dromedary camels, age (>45 years of age), and comorbidities as risk factors for infection and disease. Smoking and alcohol consumption seem to worsen such vulnerability, however, they unlikely constitute a major risk factors alone. This is one of the areas that needs further study.

The systematic inclusion of relevant animal exposure history in case ascertainment by clinicians is crucial to improve our understanding of zoonotic disease.

The availability of detailed reports of every case of MERS-CoV infection will provide valuable information to the scientific community that may be used to track, contain, and possibly eradicate this disease more effectively and efficiently. Therefore, further epidemiologic studies are needed to investigate in more detail which aspects of contact with camels or a camel product constitutes a risk factor for MERS-CoV infection.

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Chapter 3

Risk factors for primary Middle East respiratory syndrome coronavirus infection in camel workers in Qatar during 2013–2014: a case-control study

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ABSTRACT

The transmission routes and risk factors for zoonotic Middle East respiratory syndrome coronavirus (MERS-CoV) infections are still unknown. We used the World Health Organization questionnaire for MERS-CoV case-control studies to assess risk factors for human MERS-CoV seropositivity at a farm complex in Qatar. Nine camel workers with MERS-CoV antibodies and 43 workers without antibodies were included. Some camel-related activities may pose a higher risk of MERS-CoV infection, as may cross-border movements of camels, poor hand hygiene, and overnight hospital stays with respiratory complaints. The risk factors identified in this study can be used to develop infection prevention and control measures for human MERS-CoV infections.

Keywords: MERS-CoV; coronavirus; risk factors; transmission; zoonotic

BACKGROUND

In 2012, a novel coronavirus, later named “Middle East respiratory syndrome coronavirus” (MERS-CoV), was isolated from a patient with pneumonia in Saudi Arabia [1]. In 2013, the first serological evidence of dromedary camels as reservoir host species for MERS-CoV was published [2], followed by detection of highly similar viruses in dromedary camels and symptomatic humans in contact with these animals. Further support for zoonotic MERS-CoV infection was provided by the detection of MERS-CoV antibodies in camel-exposed persons, but the transmission routes and risk factors for primary, zoonotic MERS-CoV infections are still not elucidated [3].

Therefore, using the World Health Organization (WHO) questionnaire for case-control studies, we assessed risk factors for the presence of MERS-CoV antibodies in camel workers at a farm complex with circulation of MERS-CoV in camels in Qatar [4]. Previously, a cross-sectional MERS-CoV serosurvey at this farm complex revealed a 5.1% seropositivity rate among the camel workers [5]. The outcomes of this study can be used to further establish evidence-based infection prevention and control measures for primary human MERS-CoV infections.

METHODS

Study Cohort

The camel farm complex in the Dukhan area of West Qatar consists of 5 barns with approximately 6000 racing and milking camels. Each barn includes a communal dormitory for all personnel. The results of MERS-CoV-specific serologic tests of the camel workers were described in a broader Qatar-wide seroprevalence study by Reusken et al [5]. Nine camel workers with antibodies specific for MERS-CoV and 43 workers without such antibodies as previously determined by S1-based protein microarray testing were included in this study [5]. A total of 3–4 seronegative workers per seropositive worker were randomly selected, based on the proximity of their bed to a seropositive worker, their age, their sex, and the date they joined the farm. Exclusion criteria were hospital admission within 14 days before serum sampling or recent contact (ie, within 14 days) with a person confirmed to be infected with MERS-CoV or with a hospitalized patient with a respiratory illness of unknown cause. None of the selected workers refused to participate or met the exclusion criteria.

Data Collection

All study participants were subjected to the WHO questionnaire, which was slightly adapted to the local situation (Supplementary Materials).

The interviews took place in April 2014 and were completed within 1 week. The questionnaires were conducted in Arabic or Urdu by trained staff from the Qatar Ministry of Health.

Data Analysis

We compared the questionnaire results of seropositive and seronegative workers by using the Welch t test and the Fisher exact test, performed in Stata/SE 14.1 for Windows. The Mantel-Haenszel test was used for multivariate testing, performed in R-3.3.2 for Windows, with a maximum of 2 variables and with a minimum of 4 events each. When a question was left unanswered, we assumed a negative (ie, “no” or “never”) response. Likert scales were converted to binary answers (“never,” “rarely,” and “monthly” were converted to “rarely”; “weekly” and “daily” were converted to “frequently”).

Ethical Approval

The investigation was part of an official public health outbreak investigation. The joint investigation team obtained written informed consent from all participants, as well as written approvals from the Public Health Department of the Qatar Ministry of Health.

RESULTS

General Cohort Characteristics

The study subjects were all male, with a mean age of 28 years (Table 1). They originated from Bangladesh, Pakistan, Sudan, Nepal, and India. On average, they lived in Qatar for 3 years. Four of 52 (8%) smoked shisha, and 16 of 52 respondents (31%) smoked tobacco, either now or in the past. No subjects reported ravelledng disease.

A Three of 5 seropositive workers and 1 of 11 seronegative workers reported that they were former tobacco smokers.

Fifty percent (26 of 52) reported that they regularly cleaned animal housing facilities. Most subjects in this subset also indicated that they handled animal waste (25 of 26; $P < .001$) and cleaned farm equipment (15 of 26; $P < .001$; Table 2). Among all subjects, 25% (13) milked camels more than once per week, and 12% (6) frequently assisted with calvings; all of which were also involved in milking camels ($P < .001$). Thirteen percent of subjects (7) were involved in camel training.

Univariate Analysis

Regular involvement in training and herding of camels (44% of seropositive participants vs 7% of seronegative participants; $P = .01$), cleaning farm equipment (67% vs 26%; $P = .05$), and milking camels (55% vs 19%; $P = .03$) were associated with MERS-CoV seropositivity (Table 2). Workers involved in milking consumed raw camel milk (55% vs 19%; $P = .03$) and raw milk products (55% vs 19%; $P = .03$) significantly more often than workers not involved in milking, but correcting for these 2 parameters did not change the association between milking and MERS-CoV seropositivity. MERS-CoV–seropositive workers also seemed to assist with calving more often than seronegative workers, although the difference was not significant (33% vs 7%; $P = .08$).

Table 1: General Characteristics of the Study Participants with and Those without Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Infection

Characteristic	MERS-CoV–Seropositive Workers (n = 9)	MERS-CoV–Seronegative Workers (n = 43)
Male sex	9 (100)	43 (100)
Age, y	30.9 (25.4–36.4)	27.0 (25.2–28.8)
Nationality		
Bangladesh	4 (44)	18 (42)
Pakistan	2 (22)	16 (37)
Sudan	2 (22)	5 (12)
Nepal	1 (11)	3 (7)
India	0 (0)	1 (2)
Tobacco use ^a	5 (56)	11 (26)
Shisha use	1 (11)	3 (7)

Data are no. (%) of subjects or mean value (95% confidence interval).

Table 2: Selection of Possible Risk Factors for Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Antibodies: Univariate Analysis

Risk Factor	MERS-CoV– Seropositive Workers, No. (%) (n = 9)	MERS-CoV– Seronegative Workers, No. (%) (n = 43)	Pa
Primary job			
Animal care	7 (78)	28 (65)	.70
Animal training	4 (44)	3 (7)	.01
Housework	0 (0)	2 (5)	>.99
Other	3 (33)	7 (16)	.35
Frequently performed activities in past 12 mo			
Touch animals	7 (78)	27 (62)	>.99
Kiss animals	0 (0)	2 (5)	.47
Clean animal housing	6 (67)	20 (47)	.46
Handle animal waste	6 (67)	19 (45)	.28
Clean farm equipment	6 (67)	11 (26)	.05
Assist in birth of animals	3 (33)	3 (7)	.08
Milk animals	5 (55)	8 (19)	.03
Slaughter animals	0 (0)	0 (0)	>.99
Administer vaccines and/or medicines	0 (0)	1 (2)	>.99
Other animals at the farm complex			
Dogs	3 (33)	2 (5)	.03
Cats	3 (33)	23 (53)	.47
Rats	3 (33)	26 (61)	.16
Mice	3 (33)	12 (28)	.70
Chickens	0 (0)	1 (2)	>.99
Pigeons	0 (0)	1 (2)	>.99
Contact with animal waste			
Present around subjects' living quarters	4 (44)	4 (9)	.02
Touched animal waste ^c	6 (67)	21 (49)	.47
Contact with sick or dead camels			
Present around sick camels	5 (58)	17 (40)	.47

Risk Factor	MERS-CoV– Seropositive	MERS-CoV– Seronegative	Pa
	Workers, No. (%) (n = 9)	Workers, No. (%) (n = 43)	
Present around dead camels	0 (0)	9 (21)	.33
Touched sick/dead camels	1 (11)	9 (21)	.67
Participation in animal transport			
New camel at the barn	5 (55)	25 (58)	.71
Animal taken to another ravelle	4 (44)	6 (14)	.06
Personal protective equipment			
None	5 (58)	30 (70)	.45
Gloves	2 (22)	10 (23)	>.99
Coveralls	0 (0)	5 (12)	.57
Dust masks	2 (22)	6 (14)	.61
Boots or boot covers	0 (0)	4 (9)	>.99
Eye protection	1 (11)	5 (12)	>.99
Hand washing			
At mealtime	5 (56)	22 (51)	>.99
Before and after animal task	4 (44)	37 (86)	.01
Beginning and end of the day	1 (11)	16 (37)	.21
Bathroom time	5 (56)	16 (37)	.46
Rarely	2 (22)	1 (2)	.07
Consumption of animal products			
Any raw milk producte	6 (67)	26 (60)	>.99
Raw camel milk	6 (67)	20 (47)	.47
Raw cow milk	0 (0)	5 (12)	.57
Uncooked meat	0 (0)	0 (0)	>.99
Travel outside Qatar in past 6 mof	2 (22)	2 (5)	.13
Respiratory complaints in past 12 mo			
Required physician visit	3 (33)	5 (12)	.13
Required overnight hospital stay	3 (33)	1 (2)	.01
Current complaints			
Fever and cough	2 (22)	2 (5)	.13
Shortness of breath	0 (0)	0 (0)	>.99
Vomiting	2 (22)	0 (0)	.03
Diarrhea	0 (0)	0 (0)	>.99
Headache	2 (22)	0 (0)	.03

a By the Fisher exact test.

B Daily or weekly.

C All contact was with camel waste.

D Oman, United Arab Emirates, and Saudi Arabia.

E Among those who consumed any raw milk product, 94% consumed milk, and 3% consumed cheese; the type of raw milk products consumed by 3% was unknown.

F Both seropositive subjects ravelled to Saudi Arabia, 1 seronegative subject ravelled to Pakistan, and 1 seronegative subjects ravelled to Bangladesh.

Handwashing before and after animal handling was more common among seronegative workers (44% vs 86%; $P = .01$), and a greater percentage of seropositive workers indicated that they rarely washed hands, although the difference was not significant (22% vs 2%; $P = .07$). MERS-CoV–seropositive workers were also more likely to be involved in the movement of the camels in their care to other locations (44% vs 14%; $P = .06$). Of the workers who reported such movements, 3 (75%) in the seropositive group reported international movements (Saudi Arabia, United Arab Emirates, and Oman), compared with 0 seronegative workers. Two of these 3 seropositive workers also travelled to Saudi Arabia without animals in the past 6 months.

Workers with MERS-CoV antibodies were significantly more likely to report the presence of animal feces (44% vs 9%; $P = .02$) and dogs (33% vs 5%; $P = .03$) around their living quarters. Four of 5 workers who reported the presence of dogs also reported animal feces around their living quarters ($P = .001$). Other animals frequently seen at the farm complex were cats (reported by 59% of respondents), rats (reported by 55%) and mice (reported by 28%). None of the 52 study subjects indicated the presence of bats. One seropositive respondent reported drinking camel urine, although rarely, and none of the workers reported eating uncooked meat.

Significantly more seropositive workers reported an overnight stay in a hospital with respiratory complaints in the past 12 months (33% vs 2%; $P = .01$). Two seropositive workers indicated that they had fever, cough, vomiting, and headache at the time of the questionnaire, and both had been admitted to the hospital in the last 12 months. Nasal swab specimens from both workers tested negative for MERS-CoV by polymerase chain reaction analysis when they were admitted to the hospital.

DISCUSSION

We looked at possible correlations between different putative risk factors for MERS-CoV infection and the presence of MERS-CoV antibodies in camel workers. The univariate analysis revealed a correlation between the presence of MERS-CoV neutralizing antibodies in camel farm workers and cleaning farm equipment ($P = .05$), assisting in animal birth ($P = .08$), milking animals ($P = .03$), and training animals ($P = .01$). Cleaning farm equipment might represent an increased risk of MERS-CoV exposure through contact with camel saliva, feces, and/or urine on soiled equipment [6, 7]. All animal workers that were involved in calvings also milked camels more than once per week. The relative high number of workers with MERS-CoV antibodies who assisted in the birth of camels and in milking animals may be explained by intensive contact with young camels. Although newborn calves are still protected by maternal antibodies, workers who assist in animal birth or milking often remain in close contact with mothers and their calves beyond the period of maternal protection [8]. For example, milking requires the presence of suckling young camels who trigger milk flow [9].

Moreover, camel milk can contain MERS-CoV RNA [9]. An association between MERS-CoV illness and milking has been described before [10]. Camel training, which requires close contact between the animal and its trainer, also seemed to increase the risk for MERS-CoV infection in camel workers. This is in agreement with previous reports that indicated that close contact with camels can be a risk factor for MERS-CoV illness or the presence of MERS-CoV antibodies [5, 10, 11].

In our cohort, we found a greater frequency of MERS-CoV seropositivity among workers who indicated that the camels they handled had recently travelled abroad within the Arabian Peninsula. Movement of animals to and from the Dukhan farm area occur with high frequency, owing to races, trade, and breeding activities, and can contribute to continuous local MERS-CoV circulation and human exposure due to both a continuous introduction of naive animals and/or acutely infected camels. Two out of 3 workers who reported international movements of their camels also reported personal travel to Saudi Arabia in the past 6 months. It is possible that these workers were exposed to MERS-CoV during their personal travel, rather than via the camels they worked with.

Another association that was found was between the presence of MERS-CoV antibodies and overnight hospitalization because of respiratory complaints. It is possible that the admitted workers had MERS-CoV infection but were tested after virus shedding had stopped or that they acquired nosocomial MERS-CoV infection without severe symptoms.

Two of 3 seropositive workers hospitalized with respiratory complaints also reported headache, vomiting, cough, and fever at the time of the survey.

A remarkable finding was that a significantly greater percentage of seropositive workers reported the presence of dogs around their barn. The reported presence of dogs correlated strongly with the presence of animal feces (origin unknown) around the subject's living quarters ($P = .001$). There is currently no evidence of a role for dogs in MERS-CoV epidemiology. A possible explanation for the association between the presence of dogs and MERS-CoV-seropositive humans could be that dogs mechanically spread contaminated camel products (eg, feces and urine) around the farm complex.

The cohort with antibodies against MERS-CoV had a greater percentage of workers who rarely washed their hands, and washing hands before and after animal tasks appeared to have a preventive effect. Prevention of MERS-CoV infection or exposure by handwashing possibly indicates that MERS-CoV can be indirectly transmitted via fomites. Infectious MERS-CoV could still be detected on surfaces after 1 day at 30°C and in milk after 2 days at 22°C in experimental conditions [12, 13]. Contact with camel excretions and subsequent touching of mucous membranes may be an important source of infection. Nasal secretions have been shown more frequently to contain MERS-CoV and have higher viral loads as compared to camel urine, feces, and saliva [6]. Human-to-human transmission may also take place via fomites. This can explain why many individuals with a primary case of MERS-CoV infection have not reported direct camel contact and, in some cases, have reported a household member who recently visited a camel farm [10, 14].

While providing some interesting observations, this study has several limitations. First, owing to the number of respondents, the power of the study is limited. Therefore, we could only perform univariate analyses and very limited multivariate analyses to demonstrate significant associations between possible risk factors and MERS-CoV antibody presence in the respondents. Moreover, the retrospective study design may have resulted in significant recall bias among participants with regard to their and their camels' activities and health in the last 12 months. Because workers share housing and sleeping areas, MERS-CoV may have spread from human to human. This means that not all seropositive workers may have been infected directly by camels, which may affect the analysis. Last, it is possible that the MERS-CoV immunoglobulin G we detected was a result of exposure in the worker's country of origin rather than in Qatar, with MERS-CoV circulation known to exist among camels in some such countries.

A recent MERS-CoV WHO consultation on public health goals and global priority research activities called on researchers to address knowledge gaps related to, among other topics, animal reservoirs and transmission routes to humans of MERS-CoV [15]. This study adds to the understanding of MERS-CoV transmission on the human-animal interface and informs risk management. On the basis of these initial results, a larger study was initiated with the aim to include different segments of the population in Qatar.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Chapter 4

Occupational exposure to dromedaries and risk for MERS-CoV Infection, Qatar, 2013–2014

REUSKEN, C. B., FARAG, E. A., HAAGMANS, B. L., MOHRAN, K. A., GODEKE, G. J. T., RAJ, S., ALHAJRI, F., AL-MARRI, S. A., AL-ROMAIHI, H. E., AL-THANI, M., BOSCH, B. J., VAN DER EIJK, A. A., EL-SAYED, A. M., IBRAHIM, A. K., AL-MOLAWI, N., MULLER, M. A., PASHA, S. K., DROSTEN, C., ALHAJRI, M. M. & KOOPMANS, M. P. 2015. Occupational Exposure to Dromedaries and Risk for MERS-CoV Infection, Qatar, 2013-2014. *Emerg Infect Dis*, 21, 1422-5.

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ABSTRACT

We determined the presence of neutralizing antibodies to Middle East respiratory syndrome coronavirus in persons in Qatar with and without dromedary contact. Antibodies were only detected in those with contact, suggesting dromedary exposure as a risk factor for infection. Findings also showed evidence for substantial underestimation of the infection in populations at risk in Qatar.

Since Middle East respiratory syndrome coronavirus (MERS-CoV) was first detected in 2012, approximately 1,000 human infections have been reported to the World Health Organization, all linked to residence in or travel to countries on the Arabian Peninsula (1). Dromedaries (*Camelus dromedarius*) are thought to play a central role in MERS epidemiology because widespread evidence of MERS-CoV-specific antibodies and virus shedding in camels was found (2), and highly similar viruses have been detected in humans and dromedaries at the same location (3,4). These data suggest a direct zoonotic risk for MERS-CoV infection among persons in contact with camels. We describe a comparative serologic investigation in Qatar among persons with and without daily occupational exposure to dromedaries.

THE STUDY

We used 498 anonymized serum samples from persons in Qatar with and without dromedary contact (Technical Appendix) and control serum from Europe (National Institute for Public Health and the Environment, Bilthoven, the Netherlands; and University of Bonn, Bonn, Germany). Sampling in Qatar was cleared by the Ethics and Institutional Animal Care and Use Committees of the Medical Research Center, Hamad Medical Corporation (permit 2014-01-001). Samples from the Netherlands were used in accordance with the Dutch Federation of Medical Scientific Associations' code of conduct for proper use of human tissue. Samples from Germany were used in accordance with German national laws.

Of the 498 samples, 294 were from persons with daily occupational contact with dromedaries (cohorts A–D) and 204 were from persons without camel contact (cohorts E–G). Cohort A consisted of 109 healthy workers (5 camel slaughterers [subcohort A1] and 104 sheep slaughterers [A2]) at the central slaughterhouse in Doha, Qatar. All workers lived together and had contact with camels and sheep at the central animal market (CAM). Cohort B consisted of 8 CAM workers. Cohort C consisted of 22 healthy men living and working at the Al Sheehaniya barn complex near the international dromedary racing track, and cohort D consisted of 155 healthy men living and working on a dromedary farm in Dukhan, western Qatar; molecular data showed ongoing circulation of MERS-CoV in dromedaries in these locations (Technical Appendix). Cohort E consisted of 56 random samples from construction workers in Qatar. Cohort F consisted of 10 samples from persons working and living at a complex with 200 sheep barns in northern Qatar. Cohort G consisted of 138 samples for confirming specificity of the testing algorithm (66 samples from the Netherlands and Germany from persons with recent human CoV infection [subcohort G1] and 72 samples from the Netherlands obtained for routine testing from persons with suspected *Bordetella pertussis* infection [G2]).

We used microarray technology as described (3,5,6) to analyze samples for the presence of IgG reactive with MERS-CoV S1 antigen (Table). To avoid overinterpretation of data, we set the reactivity cutoff at 30,000 relative fluorescent units for subsequent analyses (6). Samples from 20 of 294 persons with camel contact were reactive; no control or noncontact samples were reactive. Among camel handlers at the Al Sheehaniya and Dukhan locations, 4 of 22 and 8 of 155, respectively, had antibodies to MERS-CoV S1. At the CAM, 1 of 8 handlers had antibodies. At the slaughterhouse location, 3 of 104 sheep slaughterers and 4 of 5 camel slaughterers were antibody-positive (Figure).

Samples from subcohort G1 (n = 66) and from all camel-contact cohorts were tested for antibodies to CoV OC43 S1, a common human CoV; all showed high seropositivity (range 89%–100%) (Figure). All 498 samples were tested for reactivity to severe acute respiratory syndrome CoV S1; none reacted (Figure).

We used a 90% plaque-reduction neutralization test (PRNT90) to confirm the presence of MERS-CoV-specific antibodies in serum samples from camel handlers. For testing,

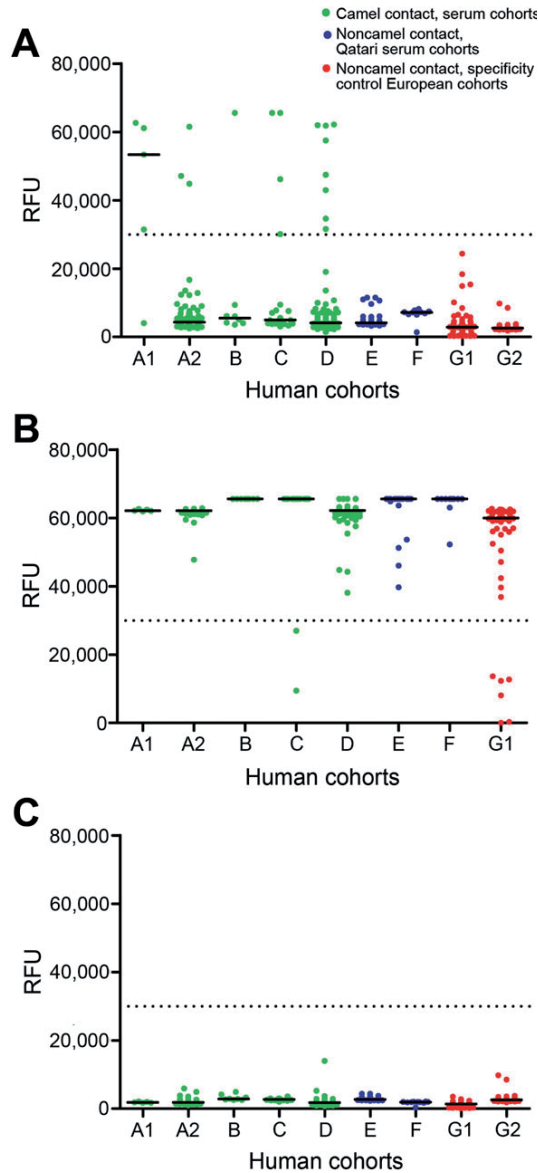


Figure: Reactivity of human serum samples, from persons with and without dromedary contact, with S1 antigens of various coronaviruses (CoVs), Qatar, 2013–2014. A) Middle East respiratory syndrome CoV S1; B) human CoV OC43 S1; C) severe acute respiratory syndrome CoV S1. Relative fluorescent units (RFU) are shown at a serum dilution of 1:20. Black lines indicate median; dotted black lines at 30,000 RFU depict cutoff for analysis. Human cohorts: A1, camel slaughterers; A2, sheep slaughterers who had contact with dromedaries and camel slaughterers; B, workers at the central animal market; C, barn workers at the international camel racing track; D, workers on camel farms; E, construction workers; F, sheep farmers; G1, persons recently infected with a common human CoV (serum samples from the Netherlands and Germany); G2, persons with suspected *Bordetella pertussis* infection (serum samples from the Netherlands).

we used the 20 samples that were reactive to MERS-CoV S1 and a random selection of nonreactive samples from camel-contact ($n = 35$) and noncontact ($n = 48$) cohorts. Results were positive for 10 of the 20 MERS-CoV S1 antibody–positive samples (reciprocal titers of 20 or 40) (Table).

All but 1 of the 35 samples from persons with camel contact who had negative S1 ELISA screening results were negative by PRNT90; the positive sample had a reciprocal titer of 20 (Table). All 48 samples from the noncontact cohorts were negative by PRNT90. This finding may indicate an underestimation of MERS-CoV seroprevalence by S1 testing. Furthermore, 6 samples from S1-positive and 2 from S1-negative persons with camel contact showed a reciprocal titer of 10, but titers of 10 were not observed in the noncontact cohorts. Five of these 8 reactive samples were also positive in a whole-virus MERS-CoV immunofluorescence assay at dilution 1:100; however, we regarded these as negative to avoid overinterpretation of data (data not shown).

CONCLUSIONS

We detected MERS-CoV neutralizing antibodies in healthy persons who had daily occupational contact with dromedaries but not in persons without such contact. Only limited evidence is available regarding the presence of MERS-CoV antibodies in the general human population or in specific population cohorts. However, an overall seroprevalence of 0.15% was found in a cross-sectional study in Saudi Arabia, and among slaughterhouse workers, neutralizing antibodies were detected in 5 of 140 participants (7).

This finding is similar to our finding among slaughterhouse workers: 7 of 109 were MERS-CoV antibody–positive. Four other studies lacked serologic evidence of MERS-CoV infection in humans with occupational exposure to dromedaries (8–11). However, only 1 of those studies documented actual MERS-CoV circulation in dromedaries during human contact, and it was concluded that MERS-CoV was not highly transmissible from camels to humans, although only 7 persons had regular contact with only 1 herd (8). On several occasions, the percentage of camels shedding MERS-CoV was high (60%) at the CAM and slaughterhouse (C.B.E.M. Reusken, unpub. data). Thus, locations with a continuous flow of dromedaries with different places of origin and different immune statuses may enable prolonged circulation of MERS-CoV and sustained exposure of dromedary handlers to the virus; in Qatar, such locations would include the CAM, slaughterhouse, and barns near the international racing tracks.

In this study, PRNT90-derived antibody titers were relatively low compared with those from earlier studies of MERS patients and dromedaries (2; B.L. Haagmans, unpub. data). The lower titers might reflect the apparent asymptomatic manifestation of MERS-CoV infection, individual differences in susceptibility, or both (2). Also, primary infections may result in a short-lived antibody peak followed by a rapid waning of antibody, depending on virus

and host properties (12), as seen in influenza A(H5N1) virus infection: antibody levels are higher in symptomatic than asymptomatic H5N1-infected persons, and antibodies wane more quickly during asymptomatic infection (13). MERS-CoV antibody kinetics and the persistence of antibodies detected by different serologic methods are not known. Such parameters are needed to estimate the force of infection on the basis of serologic data (14).

MERS-CoV-seropositive participants in this study did not report severe health problems, giving evidence for frequent unrecognized human infections. Assuming the health histories are accurate, this finding implies that the current overall MERS-CoV-associated death rate of 37.1% (1) is most likely an overestimation of the actual rate and that most infections may be asymptomatic or mild. A major issue to be resolved is whether, and to what extent, asymptomatic cases contribute to the spread of MERS-CoV; it is well recognized that variability in disease transmission exists among humans (15).

Dr. Reusken is a public health virologist at the Viroscience department of Erasmus Medical Center. Her research interests include viruses operating at the animal–human interface.

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Table: Results of MERS-CoV serologic testing of humans with and without dromedary contact, Qatar, 2013–2014*

Exposure type, cohort	Country	Serum samples tested by		
		S1 assay, no. positive/no. tested	† PRNT90, no. positive/no. tested†	
			S1-positive	S1-negative
Dromedary contact		20/294	10/20	1/35
A, slaughterhouse workers				
A1, camel slaughterers	Qatar	4/5	2/4 (40, 20)	NT
A2, sheep slaughterers (contact with camels/camel slaughterers)	Qatar	3/104	2/3 (20, 20)	1/16 (20)
B, central animal market workers				
C, barn workers at international camel racing track	Qatar	4/22	3/4 (40, 40, 20)	NT
D, camel farm workers				
No dromedary contact	Qatar	8/155	3/8 (40, 40, 20)	0/19
E, construction workers	Qatar	0/204	NA	0/48
F, sheep farmers	Qatar	0/56	NA	0/48
G, specificity controls	Qatar	0/10	NA	NT
G1, recent infection with a common hCoV				
G2, suspected infection with <i>Bordetella pertussis</i>	GER, NL	0/66	NA	NT
	NL	0/72	NA	NT

*GER, Germany; hCoV, human coronavirus; MERS-CoV, Middle East respiratory syndrome coronavirus; NA, not applicable; NL, the Netherlands; NT, not tested; PRNT90, 90% plaque-reduction neutralization test; S1, MERS-CoV S1 antigen. †Nos. in parentheses are reciprocal antibody titers in PRNT90.

TECHNICAL APPENDIX

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Description of Human Serum Cohorts

The human serum cohorts consisted of the following:

A) Anonymized serum samples from 109 healthy males (immigrants) collected in 2014 and working at the central slaughterhouse in Doha, Qatar. Only five workers exclusively work in camel slaughter in Qatar, with 3–20 years of experience (A1). All five were sampled. The other workers exclusively slaughter sheep (A2). However, all workers live together and have contact with the animals (camels, sheep) at the central animal market. The percentage of camels presented for slaughter that shed MERS-CoV was high at several occasions (1) (C.B.E.M. Reusken, unpub. data).

B) Anonymized sera from eight healthy males (originally from India, Nepal, Pakistan and Sudan) working at the Central animal market and collected in 2014. The market serves mainly camels and sheep but goats, cows, horses and donkeys as well. The market comprises – 100 pens for camels, with – 20 animals per pen. The camels originate from Australia, Iran, Oman, Pakistan, Qatar, Saudi-Arabia, Somalia, Sudan and Syria, and are sold for slaughter or use (pet, breeding, milk production) in Qatar. Animals introduced from Australia are presumed to be naive for MERS-CoV (2). This cohort has daily close contact with animals and their secretions.

C) Serum samples from 22 healthy males living and working at the Al Shahaniya barn complex near the Qatar international dromedary racing track. During the racing season from October through March, camels from around the Gulf area visit and stay together with Qatari camels at the Al Shahaniya barn complex. The barn complex consists of 749 barns with an estimated total of 14,000 camels. These persons originate from India, Nepal, Pakistan or Sudan and have daily close contact (nurturing, cleaning, grooming, training for racing) with camels residing at the barns. The sera were collected in 2013 and 2014. Published and unpublished molecular data of camel samples taken in the period 2012–2014 and the connection of the first two human cases in Qatar in 2015 with this barn complex show ongoing circulation of MERS-CoV in the complex (3–6).

D) Serum samples from 155 healthy males living and working at a camel farm in the Dukhan area, West Qatar, collected in 2013 and 2014. The farm consists of milking, breeding and racing herds with an estimated 6,000 camels (4). The camel handlers originated from India, Nepal, Pakistan or Sudan, were 20–35 years of age and had daily, intensive contact with camels (nurturing, cleaning, grooming, veterinary care, training for races). The herds at the Dukhan farm showed molecular evidence for circulation of MERS-CoV at several occasions during 2013 and 2014 (A.K. Ibrahim, unpub. data).

E) Random, anonymized serum samples collected in 2014 from 56 males working for construction companies (laborers, metal workers, guards, plumbers, crane operators,

drivers) and living in company camps in Doha, Qatar. All workers were healthy when samples were collected. The workers were <35 years of age and of Asian origin. There is no occupational contact with dromedaries, no ownership of dromedaries.

F) Anonymized serum samples from ten healthy males (immigrants) working and living at a complex with 200 sheep barns in North Qatar. At the barns >95% of the animals are sheep, other animals are goats, chickens, ducks. There is no contact with camels. Samples were collected in 2014.

G) Control group for specificity of the testing algorithm. G1) Anonymized serum samples from patients with a recent common human coronavirus (hCoV) infection (n = 66). Serum samples of 10 children, ages ranging from 9–14 months (2x hCoV-HKU1, 2x hCoV-OC43, 3x hCoV-229E and 3x hCoV-NL63 IgG positive sera) and obtained in 2001 in the Netherlands. Four anonymized hCoV-OC43 PCR positive sera from adults obtained in Germany in 2013. Fifty-four anonymized serum samples from adults obtained in Erasmus MC, the Netherlands in the period 2010–2014 and taken >2wks-<1 year after a respiratory tract sample tested positive for hCoVs using real-time RT-PCR technology (23x hCoV-OC43, 16x hCoV-229E, 15x hCoV-NL63. Serum had been collected at a later stage during hospitalization and subsequent routine visits to the out-patient clinic, the majority of these patients had recurrent health problems due to immune-deficiency, and was stored at Erasmus MC at 20°C. The study was approved by the local medical ethical committee (MEC approval: 2014–414). G2) Anonymized serum samples from 72 persons ranging in age from 0.1 year to 95.3 years sampled during 2008 for routine *Bordetella pertussis* serology in the Netherlands. This serum set represents a cohort biased toward patients with non-influenza-like respiratory symptoms (7).

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Chapter 5.1

High proportion of MERS-CoV shedding dromedaries at slaughterhouse with a potential epidemiological link to human cases, Qatar 2014

FARAG, E. A., REUSKEN, C. B., HAAGMANS, B. L., MOHRAN, K. A., STALIN RAJ, V., PAS, S. D., VOERMANS, J., SMITS, S. L., GODEKE, G. J., AL-HAJRI, M. M., ALHAJRI, F. H., AL-ROMAIHI, H. E., GHOBASHY, H., EL-MAGHRABY, M. M., EL-SAYED, A. M., AL THANI, M. H., AL-MARRI, S. & KOOPMANS, M. P. 2015. High proportion of MERS-CoV shedding dromedaries at slaughterhouse with a potential epidemiological link to human cases, Qatar 2014. *Infect Ecol Epidemiol*, 5, 28305.

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ABSTRACT

Two of the earliest Middle East respiratory syndrome (MERS) cases were men who had visited the Doha central animal market and adjoining slaughterhouse in Qatar. We show that a high proportion of camels presenting for slaughter in Qatar show evidence for nasal MERS-CoV shedding (62/105). Sequence analysis showed the circulation of at least five different virus strains at these premises, suggesting that this location is a driver of MERS-CoV circulation and a high-risk area for human exposure. No correlation between RNA loads and levels of neutralizing antibodies was observed, suggesting limited immune protection and potential for reinfection despite previous exposure.

Keywords: zoonoses, camels, MERS-CoV, respiratory infections

Dromedary camels are likely the primary source of Middle East respiratory syndrome virus (MERS-CoV) infection in humans, but further evidence is needed to support their role in zoonotic transmission. Two of the earliest diagnosed cases in Qatar were men who had visited the Doha central animal market and the adjoining central slaughterhouse (Frag, pers. comm.). Therefore, pre- and postmortem sampling was conducted on dromedary camels (n=105) at the central slaughterhouse in Doha, Qatar. Nasal, oral, and rectal swabs collected prior to slaughter were tested for the presence of MERS-CoV RNA. Most of the camels that were sampled showed evidence for MERS-CoV shedding at the time of slaughter (59%). Sequence analysis showed the circulation of at least five different virus strains at the slaughterhouse premises. An understanding of the extent and pattern of MERS-CoV shedding by dromedaries presenting for slaughter provides insight into the risks for MERS-CoV exposure of persons with occupational contact with live camels and their carcasses.

BACKGROUND

Illness associated with infection with MERS-CoV is characterized primarily by mild-to-severe respiratory complaints, most requiring hospital admission for pneumonitis or acute respiratory distress syndrome. As of June 11, 2015, ECDC has reported 1,288 laboratory-confirmed cases, including 498 deaths (1). Human-to-human transmission seems limited to family and health care settings. Overall, a large proportion of MERS cases is suspected to be a result of zoonotic transmission (1) with growing evidence for dromedary camels (*Camelus dromedarius*) as a reservoir. MERS-CoV-specific antibodies have been detected in camels across the Middle East and the African continent, suggesting a geographically widespread distribution (2). Analysis of an outbreak associated with a barn in Qatar found dromedaries and humans to be infected with nearly identical strains of MERS-CoV (3) and further support for camels as reservoir came from a study in Saudi Arabia (KSA) that found widespread circulation of different genetic variants of MERS-CoV in camels, with geographic clustering of human and camel MERS-CoV sequences (4). However, few other studies provided evidence for zoonotic transmission of MERS-CoV from camels (5). The routes of direct or indirect zoonotic transmission are yet unknown. We investigated the rate of MERS-CoV circulation in dromedaries at the slaughterhouse in Qatar, previously linked to two MERS cases in Qatar.

MERS virus shedding at slaughterhouse

A random group of 105 camels that presented for slaughter in February (n=53) and March (n=52) 2014 were sampled for MERS-CoV analysis (Table 1). Animals either had come directly from within Qatar or KSA, or had been sold through the central animal market (CM). Swabs and lymph nodes were tested for MERS-CoV RNA by internally controlled RT-PCR targeting UpE and N genes, as described (3, 6). The first camel isolate of MERS-CoV as described by Raj et al. (7) was obtained from the first group of 53 samples and among others sequences

Table 1: MERS-CoV detection in pre- and postmortem samples from camels presented for slaughter in Doha, Qatar (n=105)

Sample type	All (n=105)	0–6 months (n=41)	7–12 months (n=35)	>1 years (n=29)
Nasal	60 (61/101) ^a	63 (24/38)	74 (26/35)	39 (11/28)
Oral	23 (23/102)	18 (7/39)	35 (12/34)	14 (4/29)
Rectal	15 (15/103)	15 (6/39)	17 (6/35)	10 (3/29)
Bronchial	7 (7/101)	8 (3/38)	6 (2/34)	7 (2/29)
Lymph nodes	9 (5/53)	0 (0/19)	20 (4/20)	7 (1/14)

A Percentage positive for MERS-CoV RNA as detected by two RT-PCR targets, followed by (absolute number of samples positive/ total number tested).

generated from this group have been used to define a general MERS-CoV typing fragment (8). In total, 59% of the camels showed evidence for virus shedding in at least one type of swab at the time of slaughter (Table 1). The percentage positive samples was the highest for nasal samples, followed by oral swabs, fecal swabs, and bronchial swabs. All but one animals with virus shedding from any sample had a positive nasal swab. For saliva (oral), the percentage of positive samples was the highest for animals between 7 and 12 months of age. Lymph nodes from 53 animals were tested, yielding five positives. Approximation of the viral loads in the samples using the Ct values obtained with the UpE target showed no significant differences between types of samples and age groups (Fig. 1) It should be noted that viral loads with $\Delta Ct > 20$ were observed only in the nasal swabs and the nasal swab sample with the highest viral load was found to contain infectious virus (7).

Diversity in MERS-CoV circulation

To obtain further insight in the diversity of the viruses that circulated in dromedary camels at the slaughterhouse, MERS-CoV strains were sequenced according to a recently developed technique that enables the identification of divergent MERS-CoV types [sequences and technique in (8)]. In total, five different sequence types were identified with three different types found at both sampling moments (Table 2). Camels either came from the large Al-Shahaniya international racing complex (ASH) or from different sources elsewhere in Qatar (indicated by the initial arrow for animals 6–8 and 10–12 in (Table 2). Subsequently, they were either brought to a showing area (Al Mazad, AM), to the barns at the CM for a holding period, or immediately sent to the slaughterhouse (SH). Therefore, the sampling for animals 1–5 and 9–13 reflects MERS-CoV sequence diversity as a result of import from other regions in Qatar, whereas virus circulation at the CM more likely explains the virus diversity for animals 6–8.

Serology

Antibodies to MERS-CoV S1 were found in 100 out of 103 animals tested by micro-array technology (9). For 53 animals, antibody levels were also determined by virus neutralization

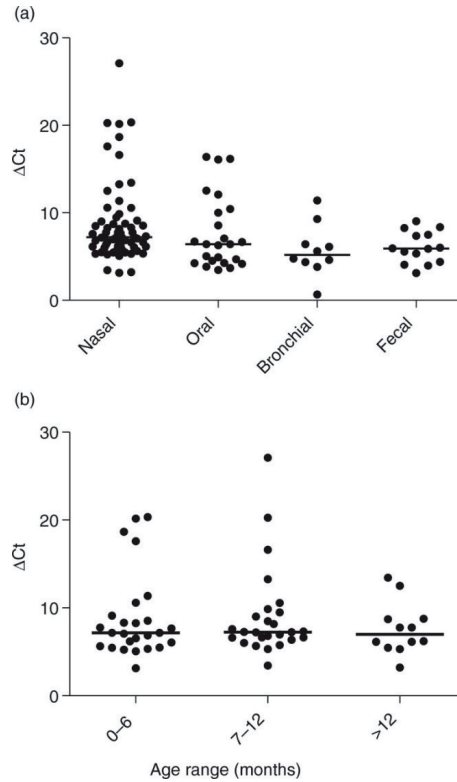


Figure 1: MERS-CoV RNA shedding by dromedary camels at the central slaughterhouse, Qatar, depicted by sample type (a) and age group for nasal swabs (b). Viral loads in samples are approximated using Ct values obtained with the Up-E target and are expressed as ΔCt ($40 - Ct_{\text{sample}}$). Black lines indicate medians.

Table 2: Summary of background information from slaughter camels for which sequences could be obtained from nasal swabs

Animal ID #	Origin	Age	Sampling moment	Sequence type
1	ASH→AM→SH	6 months	1	1
2	ASH→AM→SH	6 months	1	1
3	ASH→AM→SH	6 months	2	1
4	ASH→AM→SH	8 months	2	1
5	ASH→AM→SH	7 months	2	1
6	→CM→SH	6 months	1	2
7	→CM→SH	6 months	1	3
8	→CM→SH	8 months	1	3
9	ASH→SH	2 years	1	3
10	→AM→CM	6 months	2	3
11	→AM→CM	10 month	1	4
12	→AM→CM	6 months	2	4
13	ASH→SH	8 months	2	5

ASH=Al-Shahaniya, AM=Al Mazad, SH=slaughterhouse, CM=central market.

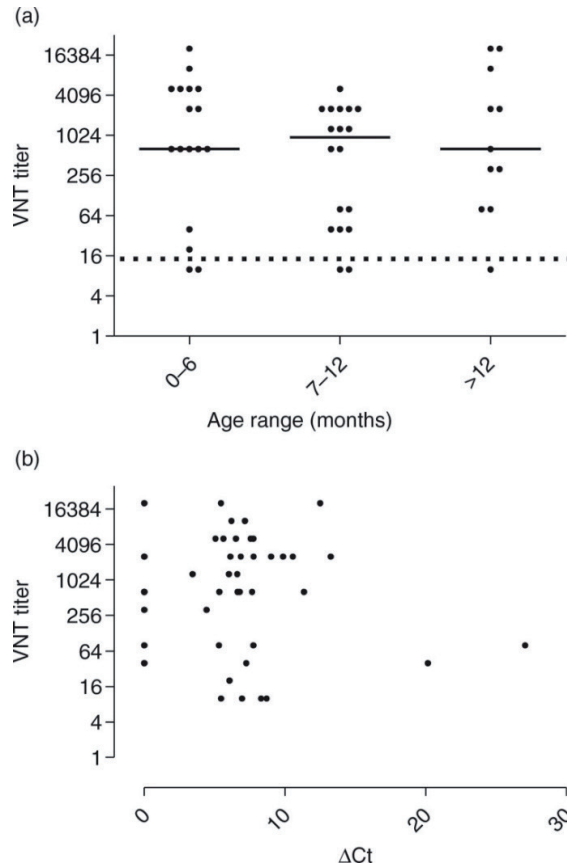


Figure 2: Reciprocal MERS-CoV-neutralizing antibodies titers by age group (a) and correlated with ΔCt (40-Ctsample) (b) for 53 camels at central slaughterhouse, Qatar.

assay as described earlier (9). Almost all animals had detectable neutralizing antibodies with no obvious age pattern and no significant difference in proportion of animals with low antibody levels (<20) (Fig. 2a). There was no correlation between antibody levels and the viral load as reflected by Ct values (Fig. 2b).

DISCUSSION

A high proportion of dromedary camels shed MERS-CoV RNA when presented for slaughter on two occasions at the central abattoir in Qatar. Co-circulation of multiple MERS-CoV variants demonstrates multiple virus introductions through flow of new animals traded into this group of animals, reflecting the virus diversity in wider Qatar, including animals

imported from Australia, the Middle East region and East Africa. This suggests that CM is a driver of MERS-CoV circulation and a high-risk site for human exposure. Indeed two cases in Qatar were linked to visits to this area, and serology data on the only five workers that exclusively work in camel slaughter in Qatar illustrated this potential burden as four of the five slaughterers had IgG antibodies specific for MERS-CoV (10)

A study at four slaughterhouses in Egypt showed an overall RNA prevalence in nasal swabs of 3.6% among 110 camels (11), which is significantly lower than in our study. A comparison of the organization of the meat markets between Egypt and Qatar could provide insight in the observed differences. The camels that are put together for a holding period of weeks prior to slaughter in Doha have a wide variety of origins with varying initial immune status, which might provide a platform for extensive virus circulation. These include naïve camels from Australia (12) and camels from areas in the Horn of Africa and the Gulf region with known differences in immune status (2, 13, 14). We observed a positivity rate in rectal swabs of 15 out of 103 animals that were analyzed (of which 61 were positive in nasal swabs). Other studies observed none to very low numbers of camels shedding MERS-CoV RNA in feces (3, 15). However, the total numbers of animals in these studies were too low to make a significant comparison with the data presented here. In the current views on MERS-CoV epidemiology, young camels (≤ 1 year) with primary infections are thought to play a bigger role in MERS-CoV transmission than older animals for which less frequent shedding is observed (4, 15) and who demonstrate higher rates of seroconversion (reviewed in (Ref. 2)). However, we observed no significant differences in MERS-CoV RNA shedding between different age groups. Moreover, the lack of correlation between viral RNA loads and levels of neutralizing antibodies in the animals suggests limited protection and potential for reinfection despite previous exposure, similar to the situation in humans with the four common human CoVs and as observed in a camel herd in KSA (15). A problem is that the time since onset of infection could not be determined as the animals did not show overt symptoms. Therefore, it remains to be determined how the kinetics of infection are. In theory, the observed shedding of virus in the presence of neutralizing antibodies could represent sampling toward the end of an infection cycle. Alternatively, the data may reflect limited mucosal immunity as has been shown for other animal coronaviruses (16). The possibility of camel vaccination has been suggested as a possible approach to controlling MERS-CoV transmission to humans. However, this may prove to be a challenging task in light of the above observations.

Given the high numbers of animals shedding these viruses in dynamic environments like the Doha market and abattoir, potential human health risks need to be considered and the implementation of management alternatives (e.g. separation of naïve animals from previously exposed animals and personal protective equipment for employees) might reduce the burden of MERS-CoV exposure to humans.

Acknowledgements

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Conflict of interest and funding

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Chapter 5.2

Isolation of MERS coronavirus from a dromedary camel, Qatar, 2014

RAJ, V. S., FARAG, E. A., REUSKEN, C. B., LAMERS, M. M., PAS, S. D., VOERMANS, J., SMITS, S. L., OSTERHAUS, A. D., AL-MAWLAWI, N., AL-ROMAIHI, H. E., ALHAJRI, M. M., EL-SAYED, A. M., MOHRAN, K. A., GHOBASHY, H., ALHAJRI, F., AL-THANI, M., AL-MARRI, S. A., EL-MAGHRABY, M. M., KOOPMANS, M. P. & HAAGMANS, B. L. 2014. Isolation of MERS coronavirus from a dromedary camel, Qatar, 2014. *Emerg Infect Dis*, 20, 1339-42.

ABSTRACT

We obtained the full genome of Middle East respiratory syndrome coronavirus (MERS-CoV) from a camel in Qatar. This virus is highly similar to the human England/Qatar 1 virus isolated in 2012. The MERS-CoV from the camel efficiently replicated in human cells, providing further evidence for the zoonotic potential of MERS-CoV from camels.

Keywords: coronavirus, MERS, camel, viruses, Qatar

BACKGROUND

Middle East respiratory syndrome coronavirus (MERS-CoV) is a novel coronavirus that can cause severe lower respiratory tract infection in humans (1,2). MERS-CoV clusters with viruses in the genus Betacoronavirus; the closest relative to this virus is bat CoVs clade 2c (3). Although bats are believed to carry different CoV ancestors, antibody reactivity against MERS-CoV has been found in serum from dromedary camels from countries within the Arabian Peninsula (4–7), Egypt (8), and the Canary Islands (4). More recently, MERS-CoVs that phylogenetically cluster with human MERS-CoVs were detected in camels from Qatar, Saudi Arabia, and Egypt (7,9–12). To further characterize MERS-CoV from camels, we screened nose swab samples from camels in Qatar .

The Study

In February 2014, nasal swab samples were collected from 53 healthy dromedary camels in Doha, Qatar. After sampling, swabs were put into tubes containing viral transport medium and stored at -80°C until shipment to the Netherlands on dry ice, as described (9).

Total nucleic acids from nasal swabs were isolated by using the MagnaPure 96 total nucleic acid isolation kit (Roche, Mannheim, Germany), and samples were tested for MERS-CoV by using 2 TaqMan assays: 1 for the envelope (upE) and 1 for the nucleocapsid gene (N), as described previously (9,13). In each assay we detected MERS-CoV RNA in a sample from an 8-month-old camel. The cycle threshold of the positive sample was 12.9 in the upE assay and 11.3 in the N assay.

For further genomic characterization, RNA was isolated from 50 μL of 1 swab sample with the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany), eluted in 60 μL water, and reverse transcribed with the Superscript III First-Strand Synthesis System (Life Technologies (Bleiswijk, the Netherlands) with random hexamers. The MERS-CoV genome was amplified by using MERS-CoV-specific overlapping primer sets as described previously (3). Amplified MERS-CoV fragments were sequenced directly on both strands by using the BigDye Terminator version 3.1 Cycle Sequencing kit on an ABI PRISM 3100 genetic analyzer (Applied Biosystems, Bleiswijk, the Netherlands). To obtain the 5' and 3' ends, we used the FirstChoice RLM-RACE kit (Ambion, Bleiswijk, the Netherlands) according to the manufacturer's protocols. Using overlapping sequence fragments, we assembled the complete MERS-CoV genome, except for 1 nt potentially missing at the 5' end.

The genome was 30,117 nt long, including 12 nt at the 3' poly A tail (MERS-CoV camel/Qatar_2_2014, GenBank accession no. KJ650098). Similar to the genome of human MERS-CoV isolates, the genome of camel MERS-CoV isolates contains 10 complete open reading frames (ORFs) (ORF 1ab, spike, ORF3, ORF4a, ORF4b, ORF5, envelope, membrane, nucleocapsid, and ORF8b), 8 transcription-regulatory sequences, and 2 terminal untranslated regions. The alignment of the camel MERS-CoV with known human MERS-CoVs, including 1 near-complete camel MERS-CoV (NRCE_HKU205) sequence, showed overall nucleotide

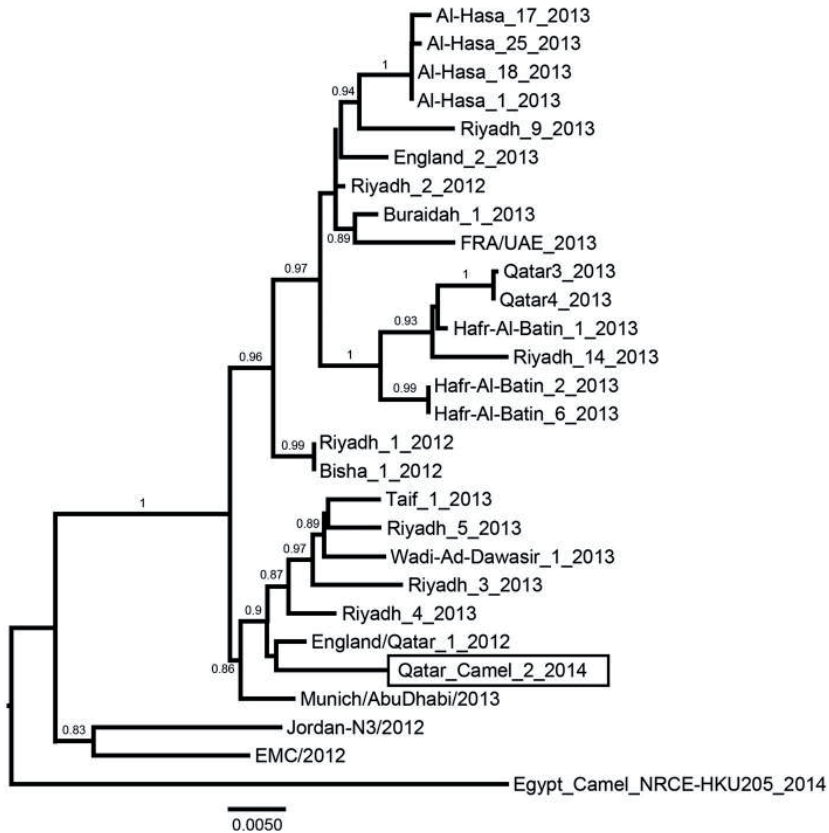


Figure 1: Phylogenetic analysis of Middle East respiratory syndrome coronaviruses (MERS-CoVs). Genome sequences of representative isolates were aligned by using ClustalW, and a phylogenetic tree was constructed by using the PhyML method in Seaview 4 (all 3 software packages can be found at <http://pbil.univ-lyon1.fr/software/seaview>) and was visualized in FigTree version 1.3.1 (<http://tree.bio.ed.ac.uk/software/figtree/>). Values at branches show the result of the approximate likelihood ratio; values <0.70 are not shown. The MERS-CoV isolated from a dromedary camel in Qatar in 2014 is depicted in a rectangle. Scale bar indicates nucleotide substitutions per site.

identities of 99.5%–99.9% between camel and human MERS-CoV isolates from different geographic regions.

Phylogenetic analysis of the complete genome clearly showed that MERS-CoV camel/Qatar_2_2014 is highly similar to human MERS-CoV; the closest relative to camel MERS-CoV was England/Qatar1 2012 (99.9% identity) (Figure 1), and it was clearly distinct from the camel MERS-CoV (99.5% identity) isolated from camels at a different location in Qatar and in Egypt (9,11). Comparison of spike protein amino acid sequences from various human and camel isolates showed that this protein is highly conserved between this camel virus and other human isolates (online Technical Appendix Table, wwwnc.cdc.gov/EID/article/20/8/14-0663-Techapp1.pdf).

In addition, most amino acid residues critical for receptor binding (14) are identical in human and camel isolates, except for L506F in England/Qatar1. The biologic relevance of this mutation has not been investigated. The presence of arginine at position 1020 in the camel virus isolate might indicate that selective pressure at this site has probably not taken place as previously postulated. The fact that a MERS-CoV from a camel is highly similar to that from a human patient who probably became infected >1 year earlier in the same region suggests that this virus is maintained within camel populations and further supports the hypothesis that MERS-CoV can be transmitted from camels to humans.

To test for the presence of infectious virus, we titrated the swab sample on Vero cells (ATCC no. CCL-81). After 48 hours, we observed cytopathic changes in cells (320 50% tissue culture infectious dose/mL). After isolation, the passage-3 virus stock was used for all subsequent experiments.

To check for adaptive mutations obtained during cell culture, we used 454 deep-sequencing technology (Roche, Indianapolis, IN, USA) to analyze the full-genome sequence as described elsewhere (3). A total of 57,655 sequence reads were obtained, of which 17,056 were specific for MERS-CoV, revealing $\approx 99.77\%$ of the virus genome. Genome coverage ranged from 1 to 2,082 reads at single nucleotide positions. Gaps or regions with coverage of <4 reads were confirmed by Sanger sequencing. When the genome of the passaged virus was aligned with the genome of the initial clinical isolate, we did not observe any mutations acquired during passaging.

To further functionally characterize this virus isolate, we subsequently inoculated human hepatoma (Huh-7) cells with MERS-CoV camel/Qatar_2_2014. After 2 days, virus-induced cytopathic effects were observed in the inoculated cell cultures (Technical Appendix Figure). In addition, a strong increase in virus titer was measured in the cell supernatant (Figure 2, panel A); produced virus could be passaged (not shown). Virus production in Huh-7 cells was blocked by preincubating camel MERS-CoV with a 1:200 dilution of serum from MERS-CoV antibody-positive camels (9) but not with seronegative camel serum (4) (Figure 2, panel A). Infection of Huh-7 cells could also be blocked by preincubation of cells with polyclonal antiserum against human DPP4 but not with control serum (Figure 2, panel A). Furthermore, transfection of nonsusceptible MDCK cells with human DPP4 (Figure 2, panel B), but not with empty vector, conferred susceptibility to infection with camel MERS-CoV (Figure 2, panel C). These data demonstrate that the MERS-CoV obtained from a dromedary camel is able to replicate in human cells and uses DPP4 as entry receptor, similar to MERS-CoV isolates obtained from human patients (15)

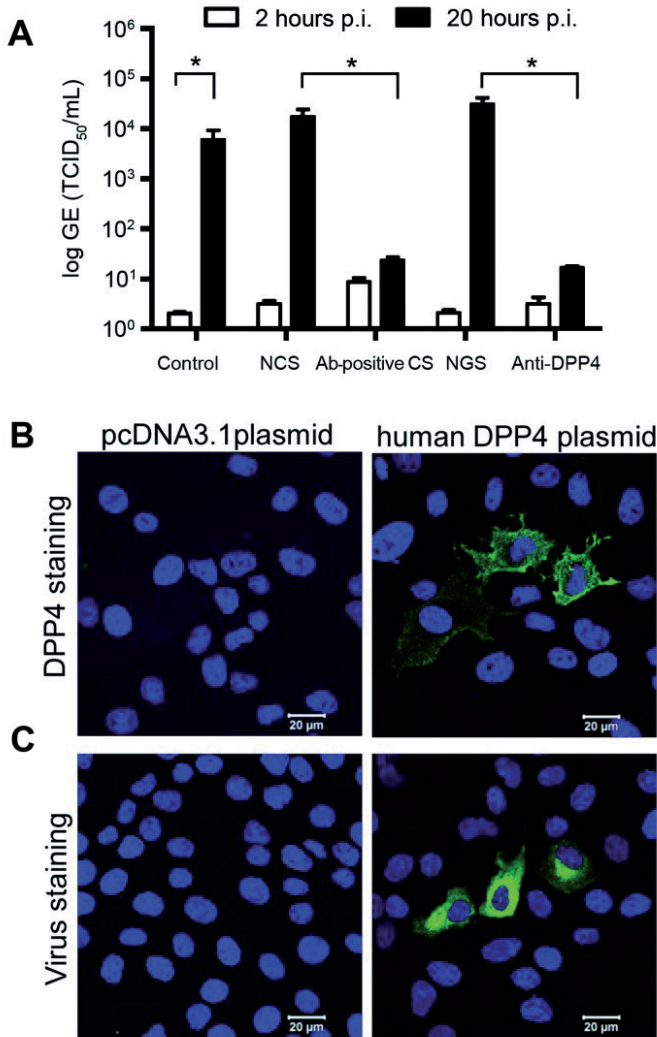


Figure 2: Middle East respiratory syndrome coronavirus (MERS-CoV) from camel replicates in human hepatoma (Huh-7) cells and uses human DPP4 as entry receptor. Huh-7 cells were inoculated with camel MERS-CoV and left for 1 h. Next, cells were washed twice, and supernatant was collected at 2 h (open bars) and 20 h (closed bars) before being tested for MERS-CoV RNA by using a TaqMan assay. We analyzed control camel MERS-CoV-infected cells, cells inoculated with camel MERS-CoV in the presence of normal camel serum (NCS), MERS-CoV-antibody positive camel serum (Ab-positive CS), normal goat serum (NGS), and anti-DPP4 polyclonal antibody-treated cells. Results are expressed as genome equivalents (GE), 50% tissue culture infective dose (TCID₅₀/mL) (A). MDCK cells transfected with plasmid-encoding human DPP4 or a control plasmid (pcDNA) were stained with polyclonal antibody against human DPP4 (B) or inoculated with camel MERS-CoV and fixed 20 h after inoculation (p.i.) and stained for viral antigen (C).

CONCLUSIONS

We isolated MERS-CoV from the nasal cavity of 1 dromedary camel and demonstrated its infectiousness. Further studies are needed to test whether camels infected at a young age are more likely than adult dromedary camels to excrete infectious virus, possibly because of the MERS-CoV seronegative status of the younger camels. In addition, our results add to recent findings that MERS-CoVs from camels and humans are nearly identical (9–11). As might be expected from the high level of conservation in the critical interacting amino acids in the receptor-binding domain of the camel and human MERS-CoV isolates (online Technical Appendix Table), we show that camel MERS-CoV can infect human Huh-7 cells by using the same entry receptor as the human MERS-CoV isolates (15). Collectively, combined with the observation that the sequence of this virus was most closely related to that of a virus from a human patient who acquired MERS-CoV in Qatar a year earlier, these data support the hypothesis that dromedary camels are a reservoir for MERS-CoV and can transmit the infection to humans. However, whether exposure of humans to camels directly can lead to human infection cannot be concluded from our data. We are not aware of a connection between the camel population sampled in this study and the patient infected with MERS-CoV England/Qatar 1. Future epidemiologic studies are needed to investigate whether contact with camels or camel products constitutes a risk factor for MERS-CoV infection.

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Biography

Dr Stalin Raj is a postdoctoral scientist at the Department of Viroscience, Erasmus Medical Center, Rotterdam, the Netherlands. His research interests are the molecular characterization and pathogenesis of emerging viruses..



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TECHNICAL APPENDIX

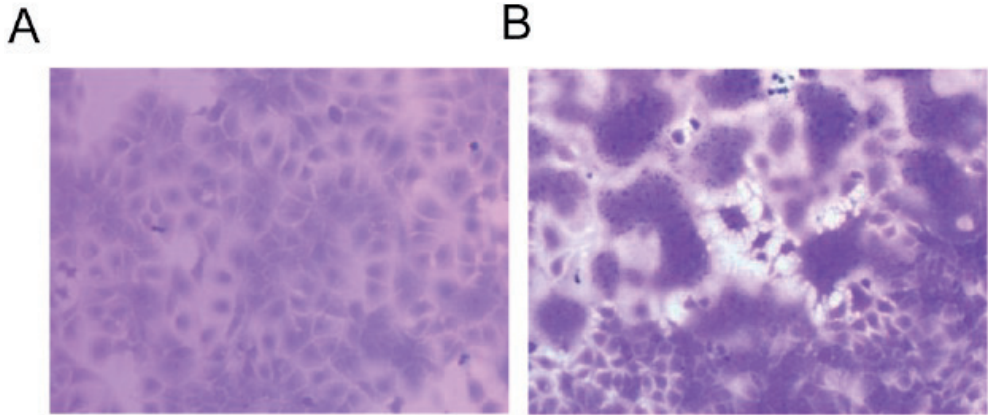
Article DOI: <http://dx.doi.org/10.3201/eid2008.140663>

Isolation of MERS Coronavirus from a Dromedary Camel, Qatar, 2014

Appendix Table. Variable amino acids in the Middle East respiratory syndrome coronavirus spike protein in different isolates

	23	26	51	94	125	158	194	201	301	431	434	460	482	506	509	534	666	696	756	886	888	914	918	1020	1083	1158	1193	1208	1227	1333	1346
EMC_2012	P	V	S	G	N	D	H	Y	R	A	I	A	A	L	D	W	E	M	E	A	S	Q	A	Q	A	A	A	Q	T	C	E
Qatar_Camel_2_2014	P	V	S	G	N	D	H	H ^a	R	A	I	A	A	L	D	W	E	M	E	A	S	Q	A	R	A	A	A	Q	T	C	E
Al-Hasa_1_2013	P	V	S	G	N	D	H	Y	R	A	I	A	A	L	D	W	E	M	E	A	S	Q	A	R	A	A	A	H	T	C	E
Al-Hasa_17_2013	P	V	S	G	N	D	H	Y	R	A	I	A	A	L	D	W	E	M	E	A	S	Q	A	R	A	A	A	H	T	C	E
Al-Hasa_18_2013	P	V	S	G	N	D	H	Y	R	A	I	A	A	L	D	W	E	M	E	A	S	Q	A	R	A	A	A	H	T	C	E
Al-Hasa_21_2013	P	V	S	G	N	D	H	Y	R	A	I	A	A	L	D	W	E	M	E	A	S	Q	A	R	S	A	A	H	T	C	E
Al-Hasa_25_2013	P	V	S	G	N	D	H	Y	R	A	I	A	A	L	D	W	E	M	E	A	S	Q	A	R	A	A	A	H	T	C	E
Bisha_1_2012	P	V	S	G	N	D	H	Y	R	A	I	A	L	G	W	E	M	E	A	S	Q	A	R	A	A	A	Q	T	C	E	
Buraidah_1_2013	P	V	S	G	N	D	H	Y	R	A	I	A	A	L	D	W	E	M	E	A	S	Q	A	R	A	A	A	Q	I	C	E
Egypt_Camel_HKU205_2014	L	A	S	G	N	D	Y	Y	R	A	S	A	A	L	D	W	K	T	Q	S	I	Q	S	Q	A	S	A	Q	T	R	E
England/Qatar_1_2012	P	V	S	G	N	D	H	Y	R	A	I	A	A	F ^b	D	W	E	M	E	A	S	Q	A	H	A	A	A	Q	T	C	E
England_2_2013	P	V	S	G	N	D	H	Y	R	A	I	A	A	L	D	W	E	M	E	A	S	Q	A	R	A	A	A	Q	T	C	E
FRA/UAE_2013	P	V	S	G	Y	D	H	Y	R	A	I	A	A	L	D	W	E	M	E	A	S	H	A	R	A	A	S	Q	T	C	E
Hafr-Al-Batin_1_2013	P	V	S	G	N	Y	H	Y	R	A	I	A	A	L	D	W	E	M	E	A	S	Q	A	R	A	A	A	Q	T	C	E
Hafr-Al-Batin_2_2013	P	V	S	G	N	Y	H	Y	R	A	I	A	A	L	D	W	E	M	E	A	S	Q	A	R	A	A	A	Q	T	C	E
Hafr-Al-Batin_6_2013	P	V	S	G	N	Y	H	Y	R	A	I	A	A	L	D	W	E	M	E	A	S	Q	A	R	A	A	A	Q	T	C	E
Jordan-N3/2012	P	V	S	V	N	D	Y	Y	L	A	I	A	A	L	D	W	E	M	E	A	S	Q	A	Q	A	S	A	Q	T	C	E
Munich/AbuDhabi/2013	P	I	S	G	N	D	H	Y	R	A	I	A	A	L	D	W	E	M	E	A	S	Q	A	H	A	A	A	Q	T	C	E
Qatar3_2013	P	V	S	G	N	D	H	Y	R	A	I	F	A	L	D	W	E	M	E	A	S	Q	A	R	A	A	A	Q	T	C	E
Qatar4_2013	P	V	S	G	N	D	H	Y	R	A	I	F	A	L	D	W	E	M	E	A	S	Q	A	R	A	A	A	Q	T	C	E
Riyadh_1_2012	P	V	S	G	N	D	H	Y	R	A	I	A	A	L	G	W	E	M	E	A	S	Q	A	R	A	A	A	Q	T	C	E
Riyadh_14_2013	P	V	S	G	N	D	H	Y	R	A	I	A	A	L	D	W	E	M	E	A	S	Q	A	R	A	A	A	Q	T	C	E
Riyadh_2_2012	P	V	S	G	N	D	H	Y	R	A	I	A	A	L	D	A	E	M	E	A	S	Q	A	R	A	A	A	Q	T	C	E
Riyadh_3_2013	P	V	S	G	N	D	H	Y	R	A	I	A	A	L	D	W	E	M	E	A	S	Q	A	H	A	A	A	Q	T	C	E
Riyadh_4_2013	P	V	S	G	N	D	H	Y	R	A	I	A	A	L	D	W	E	M	E	A	S	Q	A	H	A	A	A	Q	T	C	E
Riyadh_5_2013	P	V	F	G	N	D	H	Y	R	A	I	A	A	L	D	W	E	M	E	A	S	Q	A	H	A	A	A	Q	T	C	E
Riyadh_9_2013	P	V	S	G	N	D	H	Y	R	P	I	A	V	L	D	W	E	M	E	A	S	Q	A	R	A	A	A	Q	T	C	E
Taif_1_2013	P	V	S	G	N	D	H	Y	R	A	I	A	A	L	D	W	E	M	E	A	S	Q	A	H	A	A	A	Q	T	C	E
Wadi-Ad-Dawasir_1_2013	P	V	S	G	N	D	H	Y	R	A	I	A	A	L	D	W	E	M	E	A	S	Q	A	H	A	A	A	Q	T	C	Q
Consensus	P	V	S	G	N	D	H	Y	R	A	I	A	A	L	D	W	E	M	E	A	S	Q	A	R	A	A	A	Q	T	C	E

^aAmino acid residues in the Middle East respiratory syndrome (MERS-CoV) spike protein that differ from the EMC/2012 isolate are displayed in red.^bCritical amino acid in binding to DPP4 receptor, displayed in green.



Appendix Figure. Middle East respiratory syndrome coronavirus (MERS-CoV) from camel replicates in human hepatoma (Huh-7). Mock-inoculated cells (A) or cells inoculated with camel MERS-CoV (B) were fixed 40 hours after inoculation and stained with crystal violet.



Chapter 5.3

Failure to detect MERS-CoV RNA in urine of naturally infected dromedary camels

FARAG, E. A., HAAGMANS, B. L., AL-ROMAIHI, H., MOHRAN, K., HAROUN, M., EL-SAYED, A. M., KOOPMANS, M., ALHAJRI, M. & REUSKEN, C. 2019b. Failure to detect MERS-CoV RNA in urine of naturally infected dromedary camels. *Zoonoses Public Health*, 66, 437-438.

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LETTER TO THE EDITOR

Dromedaries (*Camelus dromedarius*) are reservoirs for zoonotic transmission of Middle-East respiratory syndrome coronavirus (MERS-CoV) but even six years after its discovery the transmission route(s) of MERS-CoV from dromedaries to humans has not been fully elucidated.

The World Health Organization recommends that groups at high risk for severe MERS should avoid contact with dromedary camels, consumption of raw camel milk or camel urine, as well as eating meat that has not been properly cooked (WHO, 2018). Although MERS-CoV RNA has been detected in dromedary camel nasal secretions, saliva, feces and milk (Farag, 2014; Reusken, 2014; Haagmans, 2015), and in human urine samples (Corman, 2016), so far no evidence has been obtained for the presence of the virus in dromedary urine (Adney, 2014; Ali, 2017). However, it has been speculated that, amongst others, collection and consumption of urine from acutely infected camels might create circumstances for cross-species transmission event (Gossner, 2016; MacKay, 2015). Dromedary camel urine plays an ancient traditional and religious role in daily life in the Middle East and North Africa region as well as in parts of Asia. Camel urine is believed to have therapeutic effects in the treatment of cancer, diabetes, certain infectious and cardiovascular diseases as well as in the treatment of hair and skin problems. Hence, fresh urine is consumed, used to wash body and hair, and is a component in ointments (Gader, 2016; Alkhamees, 2017); e.g. it has been described that Bedouins in the Middle-East have a daily consumption of 100 ml camel urine while a study among 156 Saudi cancer patients showed that 15.7% drank camel urine (Al-Yousef, 2012; Abuelgasim, 2018).

Here, we investigated the presence of MERS-CoV RNA and specific antibodies in urine of camels that were offered for slaughter at the central slaughterhouse in Doha, Qatar in March 2014. Qatar has reported 19 MERS cases as of August 2018 (WHO, 2018). Camels at the Doha slaughterhouse were shown to have a high prevalence of MERS-CoV RNA shedding. A previous study showed that 59% of the camels had evidence for virus shedding in at least one type of swab at the time of slaughter (Farag, 2014). Urine from 23 camels, aged 4 months to 10 years (median 6 months), was collected aseptically post-slaughter from intact bladders using 20 ml syringes. The collected urine was stored at -80°C until RNA extraction as described before (Reusken, 2014). The urine was analysed for the presence of MERS-CoV using a screening RT-PCR targeting the UpE region and a confirmatory RT-PCR targeting the N-gene as described before (Farag, 2014). We interpreted the urine results in the context of the presence of MERS-CoV RNA and antibodies in each respectively same-time collected nasal swab and serum sample (data in Farag, 2014). In none of the 23 urine samples MERS-CoV RNA could be detected while of the corresponding 23 nasal swabs 11 camels tested positive using both tests. The same urine samples were analysed for the presence of MERS-CoV specific antibodies using micro-array technology (Reusken, 2013a,b). We found MERS-CoV specific antibodies in 16 of 23 urine samples while all

camels showed such evidence for a (previous) MERS-CoV infection in serum. Based on the observed relative fluorescence the overall reactivity of the antibodies in sera was higher than of those present in urine (Data not shown). The specificity of the antibodies detected in the serum samples was confirmed by virus neutralization (Reusken, 2013b).

Although 11 camels showed evidence for an acute MERS-CoV infection at the time of urine sampling and all camels showed evidence for a (past) infection based on the presence of antibodies in serum, we found no evidence for shedding of MERS-CoV RNA in urine. These results are in line with data obtained from another field study and experimentally infected dromedaries, indicating the absence of MERS-CoV in camel urine (Adney, 2014; Ali, 2017). It should be noted that failure to detect the virus in urine in the former field investigation (Ali, 2017), in contrast to our study, was not linked to dromedaries with MERS-CoV RNA in their nasal swab. Together the studies imply the absence of a role of camel urine in MERS-CoV transmission to humans. However, to establish unequivocally that urine does not play a role in zoonotic transmission of MERS-CoV a large cohort study may be needed including animals of different age groups and at different stages of infection with simultaneous and longitudinal sampling of urine, serum and swabs. In the absence of results of such a systematic study, prudence towards consumption of raw camel urine is still indicated.

Conflict of Interest

The authors declare that there is no conflict of interest

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Chapter 5.4

Rapid communications Middle East respiratory syndrome coronavirus (MERS- CoV) RNA and neutralising antibodies in milk collected according to local customs from dromedary camels, Qatar, April 2014

REUSKEN, C. B., FARAG, E. A., JONGES, M., GODEKE, G. J., EL-SAYED, A. M., PAS, S. D., RAJ, V. S., MOHRAN, K. A., MOUSSA, H. A., GHOBASHY, H., ALHAJRI, F., IBRAHIM, A. K., BOSCH, B. J., PASHA, S. K., AL-ROMAIHI, H. E., AL-THANI, M., AL-MARRI, S. A., ALHAJRI, M. M., HAAGMANS, B. L. & KOOPMANS, M. P. 2014. Middle East respiratory syndrome coronavirus (MERS-CoV) RNA and neutralising antibodies in milk collected according to local customs from dromedary camels, Qatar, April 2014. *Euro Surveill*, 19. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20829>

ABSTRACT

Antibodies to Middle East respiratory syndrome coronavirus (MERS-CoV) were detected in serum and milk collected according to local customs from 33 camels in Qatar, April 2014. At one location, evidence for active virus shedding in nasal secretions and/or faeces was observed for 7/12 camels; viral RNA was detected in milk of five of these seven camels. The presence of MERS-CoV RNA in milk of camels actively shedding the virus warrants measures to prevent putative food-borne transmission of MERS-CoV. In April 2014, serum, nasal swabs and rectal swabs were taken from 33 milking dromedary camels at two locations in Qatar (Al Sheehaniya and Dukhan), areas with known Middle East respiratory syndrome coronavirus (MERS-CoV) circulation in camels [1] and data not shown. In addition, milk was collected from these animals according to local customs. Serum samples and milk were tested for the presence of MERS-CoV-specific antibodies by protein microarray, with confirmation by virus neutralisation. Swabs and milk were tested for the presence of MERS-CoV RNA by real-time reverse transcription (RT)-PCR testing for multiple genomic targets. Antibodies to MERS-CoV were detected in serum and milk from all camels at both locations. At the Dukhan location, none of the 21 animals tested was actively shedding viral RNA from the nose and/or in faeces and no evidence for the presence of MERS-CoV RNA in milk was observed. At the Al Sheehaniya location, evidence for active virus shedding was observed for seven of the 12 camels tested. Viral RNA was detected in milk of five of the seven camels with active virus shedding.

BACKGROUND

In 2012, MERS-CoV was identified in patients with severe respiratory illness in the Middle East [2]. As of 11 June 2014, a total of 683 cases including 204 deaths have been reported to the World Health Organization (WHO) [3]. All cases have had an epidemiological link to the Middle East, with confirmed cases in Iran, Jordan, Kuwait, Lebanon, Oman, Saudi Arabia, Qatar, United Arab Emirates and Yemen. Human-to-human transmission seems limited to family and healthcare settings and is assumed to have contributed to the recent upsurge of cases [4]. Overall, however, a large proportion of cases of MERS-CoV infection is community acquired, with suspected zoonotic transmission, although the extent thereof remains to be determined [5]. Dromedary camels (*Camelus dromedarius*) are the prime suspects to serve as an animal reservoir for MERS-CoV, although alternative sources remain possible [6-11].

In August 2013, dromedary camels were implicated for the first time as a possible source of the virus leading to human infection on the basis of the presence of MERS- CoV neutralising antibodies in dromedaries from Oman and the Canary Islands of Spain [6]. Since then, MERS- CoV-specific antibodies have been detected in camels across the Middle East and in several African countries [7-9]. Analysis of an outbreak of MERS-CoV infection in humans associated with a barn in Qatar in October 2013 found dromedaries and humans to be infected with nearly identical strains of MERS-CoV [1] and the virus was isolated from dromedaries shortly after [10]. Further support for camels as a reservoir came from a study in Saudi Arabia that found widespread circulation of different genetic variants of MERS-CoV in camels, and antibodies in samples taken since the early 90s [11].

Although camels are suspected to be the primary source of MERS-CoV leading to human infection, the routes of direct or indirect zoonotic transmission remain unknown. A possible route might be food-borne transmission through consumption of raw camel milk or undercooked meat. Here we report on our investigations into virus shedding of milking camels, in relation to the presence of MERS-CoV RNA in milk, as a first assessment of a potential role of consumption of raw camel milk in MERS-CoV transmission.

Analysis of dromedary serum, milk, nasal and rectal swabs

Sample collection

In April 2014, serum, nasal swabs, rectal swabs and milk were collected from 12 dromedary camels in three barns at the Al Sheehaniya barn complex and 21 dromedary camels from a milking herd in the Dukhan area, Qatar. The milking camels at the barns at Al Sheehaniya were kept together with racing camels that have regular contact with camels outside the barn at practice and racing events. Barn 1 held 22 racing and nine milking camels. Barn 2 held 18 racing and four milking camels, while Barn 3 held 15 racing and three milking camels. Each milking camel (dam) had their calf present. The age range of the calves was three to eight months (Table).

Table: Middle East respiratory syndrome coronavirus (MERS-CoV) analysis of serum, nasal and rectal swabs and milk of dairy dromedary camels, Al Sheehaniya , Qatar, April 2014 (n=12)

Barn number	Camel dama number	Age camel dam (years)	Age calf (months)	Real-time reverse transcription-PCRb						Serology				
				Nasal swab	Rectal swab	Whole milk	Milk fat	Skimmed milk	Cell pellet	Milk totalc	Serumd	Milkd	Serume	Milke
1	1	8	3	-	-	-	-	-	-	-	+	+	≥1,280	80
	2	7	4	+	-	+	-	-	-	+	+	+	640	Eq.
	3	10	4	-	+	-	-	-	-	-	+	+	≥1,280	80
	4	8	5	+	-	-	-	-	-	-	+	+	≥1,280	Eq.
	5	7	6	+	+	+	-	+	+	+	+	+	≥1,280	40
	6	9	7	+	-	+	-	+	+	+	+	+	≥1,280	40
	7	9	7	+	-	+	-	+	+	+	+	+	≥1,280	40
2	8	10	5	-	+	+	-	+	+	+	+	+	≥1,280	40
	9	8	3	-	-	-	-	-	-	-	+	+	≥1,280	40
3	10	15	8	-	-	-	-	-	-	-	+	+	≥1,280	NT
	11	12	5	-	-	-	-	-	-	-	+	+	≥1,280	20
	12	10	7	-	-	-	-	-	-	-	+	+	≥1,280	80
Total number positive	NA	NA	NA	5	3	5	0	4	4	5	12	12	12	9

Eq.: equivocal (titre between ≥ 5 and < 20); NA: not applicable; NT: not tested due to lack of sample. A dash represents that the test was negative.

a A dam is the femal parent of a livestock animal.

b A sample is considered PCR positive for MERS-CoV when > 2 targets (UpE, Orf1a and/or N) are reactive.

c Summary results of whole milk, milk fat, skimmed milk and cell pellet.

d Serology based on MERS-CoV S1 protein-microarray. Cut-off value 4,000 relative mean fluorescent intensity.

e Serology based on MERS-CoV neutralisation assay. Starting dilution 1:5. Neutralising antibody titres are shown.

The herd in the Dukhan area was in a secluded area far from other animals. The age range of the calves was three to seven months. Both locations had known circulation of MERS-CoV in dromedaries at the end of 2013/beginning of 2014 [1] and data not shown.

No samples were collected from the calves. Serum and swabs from the dams were collected wearing a disposable gown, gloves, goggles and FFP2 mask, as described [1]. Milk was collected according to local customs as follows: dromedary calves were not weaned after delivery but kept at the farm in paddocks adjacent to their dams throughout lactation. Dams were reunited with their calf to trigger milk production. Once milk production was initiated, the milk samples were collected by the camel owner or handler according to regional customs. No specific hygienic precautions were taken (Figure). All samples were stored at -80°C until shipment to the Netherlands on dry ice. All sera and swabs were shipped in agreement with Dutch import regulations for animal samples from foot-and-



Figure: Milking camels according to local customs, Al Sheehaniya barn complex, Qatar, April 2014 Milk production is triggered by the calf: the calf is then set aside and the milk is collected. Photographs by E. Farag.

mouth disease-endemic regions and stored and handled in a biosafety level 3 laboratory until inactivation by incubation for 4 hours at 56 °C or addition of lysis buffer, respectively.

Sample testing

Total nucleic acids from swabs were isolated using an automated MagNAPure 96 extraction with the total nucleic acid isolation kit (Roche, Mannheim, Germany). Swabs were tested for MERS-CoV RNA by internally controlled real-time RT-PCR targeting UpE and N genes, as described [1,12]. Initial observations of reduced nucleic acid recovery when whole milk was extracted using routine protocols for clinical samples triggered us to test milk fractions, besides whole milk, for putative increase of sensitivity [13,14]. Total RNA was manually extracted from whole milk, skimmed milk, cellular pellet and cream components of milk samples using the High Pure RNA isolation kit (Roche, Mannheim, Germany). Extracts of whole milk and milk fractions were tested for MERS-CoV RNA by internally controlled real-time RT-PCR targeting Orf1A and UpE genes, as described [1, 12].

According to international consensus, samples were considered positive for MERS-CoV RNA when at least two different targets were reactive [15]. At Al Sheehaniya, seven of the 12 camels tested were actively shedding viral RNA from the nose (n=5) and/or faeces (n=3) with threshold cycle (Ct) values ranging between 23.0 and 29.7. Overall, milk obtained from five of the seven virus-shedding animals demonstrated presence of MERS-CoV RNA (Table) with Ct values ranging from 29.2 to 37.9. Sequence analysis of the PCR products from the milk fraction with the highest viral load confirmed the presence of MERS-CoV (data not shown). At the Dukhan location, none of the 21 animals tested was actively shedding viral RNA and no evidence for the presence of MERS-CoV RNA in milk was obtained (data not shown). Milk fractions of bulk milk collected from dairy dromedaries in the Netherlands tested negative for MERS-CoV RNA (data not shown). Serum and milk samples were tested for the presence of IgG antibodies reacting with MERS-CoV (residues 1–747), severe acute respiratory syndrome (SARS)-CoV (residues 1–676) and human coronavirus (HCoV)-OC43

(residues 1–760) spike domain S1 antigens using extensively validated protein-microarray technology, as described [6,16-18]. HCoV-OC43 S1 was used as proxy for bovine CoV (BCoV), which is known to circulate commonly in dromedaries [19,20]. All serum and milk samples from Al Sheehaniya and the Dukhan location had MERS-CoV S1 binding antibodies (Table and data not shown). Confirmation of array results from Al Sheehaniya was done by MERS-CoV neutralisation assays, as described [6]. Neutralising antibody titres varied between 640 and $\geq 1,280$ for serum and between 10 and 80 for milk with 9 out of 11 having titres fourfold above the starting dilution of 1:5 (Table). Control serum (n=3) and bulk milk collected from dairy dromedaries in the Netherlands were negative (data not shown). All serum and milk samples from both locations in Qatar and the Netherlands reacted with HCoV-OC43 S1 confirming common circulation of BCoV in camelids. All samples tested negative for SARS-CoV (data not shown). To gain insight into possible faecal contamination of the milk samples, the samples were analysed for the presence of *Escherichia coli* by a quantitative PCR based on the *E. coli uidA* gene, with a limit of quantification of <103 genome copies per ml [21]. The presence of *E. coli* was not consistently detected in repeated testing (data not shown).

DISCUSSION

Raw milk from dromedaries has been consumed by humans for thousands of years and is thought to have healing properties when consumed ‘hot’, directly out of the udder [22]. Nowadays, dromedaries are still an important source of milk in rural areas of arid countries such as Qatar and other countries in the Middle East and parts of Africa [23].

Food-borne transmission is a putative route of zoonotic transmission of MERS-CoV that needs further investigation. Recent data demonstrated that MERS-CoV experimentally introduced into camel milk can survive for up to 72 hours at 4 °C and 22 °C and it has been suggested that consumption of MERS-CoV-containing milk might result in introduction of the virus into the oral cavity and subsequent infection of the lower respiratory tract [24].

Here, we detected the presence of MERS-CoV RNA in five milk samples collected from seven animals shedding MERS-CoV from the nose and/or feces at Al Sheehaniya. Although shedding of infectious virus in ruminant milk and infection of humans due to the consumption of raw milk have been described for several viruses [25,26], it cannot be concluded from our data that this holds true for MERS-CoV as well. The milk samples were collected according to local customs in which camel udders are not normally cleaned before milking and hygienic conditions are such that udders and milk can be contaminated with nasal secretions or faeces from the camel, saliva of the calves, which are allowed to suckle prior to milking to initiate the milk flow, or dirt from the bowl or the hands of the milker. Additional studies under controlled hygienic conditions are ongoing to determine whether MERS-CoV replicates in the udder or could be introduced as contaminant during

the milking process. It remains to be seen if the results reflect the presence of infectious virus in the milk samples. The RNA loads in the milk samples were too low to attempt virus isolation; we have observed that samples containing MERS-CoV RNA with Ct values >30 in general do not contain infectious virus particles. Experiments aiming at determining the amount of infectious virus present in milk samples such as those collected in our study should be conducted locally, avoiding detrimental effects of shipment and freeze-thaw cycles on virus viability. In addition, the presence of substantial levels of MERS-CoV neutralising antibodies in the milk samples might neutralise any infectious virus present during *in vitro* testing, which may differ from the *in vivo* situation, particularly if the virus is resistant to gastric juice and passage of infectious virus through the stomach occurs [27]. Nevertheless, it can be concluded that the presence of MERS-CoV RNA in raw milk as consumed locally might represent a source for zoonotic transmission of MERS-CoV and prudence is called for. Munster et al. showed that heat treatment (30 minutes at 63 °C) of MERS-CoV-containing camel milk reduced levels of infectious virus below detection level [24]. Boiling milk before consumption could be an easy, achievable local measure to prevent transmission and to preserve consumption of camel milk.

An interesting observation is the difference in virus shedding between the herds at Al Sheehaniya and Dukhan (7/12 and 0/21, respectively) although virus circulation had been detected in the Dukhan location earlier (data not shown). While the current study provides only a snapshot, it suggests that herd management practices may influence virus circulation. In addition, the nasal and/or faecal shedding of MERS-CoV by animals with high levels of neutralising antibodies suggests that the presence of antibodies does not confer sterilising immunity.

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Conflict of interest

None declared.



Authors' contributions

CR: coordination of the study in the Netherlands, assisted in designing the study, analysed data, wrote manuscript.

EF: coordination of the study in Qatar, assisted in designing the study, read and revised manuscript. MJ: protocol development, performed laboratory testing, analysed data, read and revised manuscript. GJG: performed laboratory testing, analysed data, read and revised manuscript. AES: field work Qatar, read and revised manuscript. SP: performed laboratory testing, analysed data, read and revised manuscript. VSR: performed laboratory testing, analysed data, read and revised manuscript. KM: field work Qatar, read and revised manuscript. HAM: performed laboratory testing, analysed data, read and revised manuscript. HG: read and revised manuscript. FAH: read and revised manuscript. AI: field work Qatar, read and revised manuscript. BJB: design antigen production, provided antigens, read and revised the manuscript.

HAR: read and revised manuscript. SKP: read and revised manuscript. MAT: read and revised manuscript. SAM: read and revised manuscript. MAH: overall coordination collaboration Qatar-the Netherlands, assisted in designing the study, read and revised manuscript. BH: data analysis, assisted in designing the study, read and revised manuscript. MK: overall coordination collaboration Qatar-the Netherlands; assisted in designing the study, data analysis, read and revised manuscript.

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Chapter 6

Qatar experience on One Health approach for Middle-East respiratory syndrome coronavirus, 2012–2017: A viewpoint

FARAG, E., NOUR, M., ISLAM, M. M., MUSTAFA, A., KHALID, M., SIKKEMA, R. S., ALHAJRI, F., BU-SAYAA, A., HAROUN, M., VAN KERKHOVE, M. D., ELKHOLY, A., MALIK, S. M. R., REUSKEN, C., KOOPMANS, M. & ALHAJRI, M. M. 2019a. Qatar experience on One Health approach for middle-east respiratory syndrome coronavirus, 2012–2017: A viewpoint. *One Health*, 7, 100090.

ABSTRACT

The emergence of the Middle East Respiratory Syndrome Corona Virus (MERS-CoV) in the Middle East in 2012 was associated with an overwhelming uncertainty about its epidemiological and clinical characteristics. Once dromedary camels (*Camelus dromedarius*) was found to be the natural reservoir of the virus, the public health systems across the Arabian Peninsula encountered an unprecedented pressure to control its transmission. This view point describes how the One-Health approach was used in Qatar to manage the MERS-CoV outbreak during the period 2012-2017.

One-Health focuses on the association between the human, animals and environment sectors for total health and wellbeing of these three sectors. To manage the MERS outbreak in Qatar through a One-Health approach, the Qatar National Outbreak Control Taskforce (OCT) was reactivated in November 2012. The animal health sector was invited to join the OCT. Later on, technical expertise was requested from the WHO, FAO, CDC, EMC, and PHE. Subsequently, a comprehensive One-Health roadmap was delivered through leadership and coordination; surveillance and investigation; epidemiological studies and increase of local diagnostic capacity.

The joint OCT, once trained had easy access to allocated resources and high risk areas to provide more evidence on the potential source of the virus and to investigate all reported cases within 24-48 hours. Lack of sufficient technical guidance on veterinary surveillance and poor risk perception among the vulnerable population constituted major obstacles to maintain systematic One-Health performance.

Keywords: One-Health, MERS-CoV, Qatar

THE PROBLEM

The emergence of the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in the Middle East in 2012 [1] has remained a public health concern particularly in the Arabian Peninsula till the present time. Dromedary camels (*Camelus dromedarius*) have been found to be the natural reservoir from which the viral spill-over to humans can occur. Camels show no or minor clinical signs if infected with MERS-CoV [2]. As of the end of February 2019, 2,374 laboratory confirmed human cases worldwide, including 823 associated deaths (case-fatality rate: 34.6%), have been reported. The majority of these cases were reported from Saudi Arabia (1983 cases, including 745 related deaths) [3]. In Qatar, a total of 24 human cases have been reported along with 8 related deaths. At the beginning of the MERS outbreak in 2012, the lack of knowledge, particularly on the mode and speed of transmission of this novel virus, challenged the healthcare systems in Qatar as well as the entire Gulf region, amid fears that transmission could readily happen between humans. The unfamiliarity of the responding agencies of the Gulf Cooperation Council (GCC) with such an extraordinary threat heightened the concerns of the affected communities in Qatar as MERS cases continued to be reported from all neighbouring GCC countries. Moreover, the GCC countries are characterised with intensive movement of people and camels across the borders.

An eager race was started to establish the epidemiology of the disease. The epidemiological link of MERS-CoV with camels was revealed in October, 2013. Two patients had frequent contact with animals, including camels, and had no history of travel outside Qatar in the two weeks before they became ill. MERS-CoV was detected from nasal swabs of three camels with which the patients had contact [4]. A huge pressure was placed on the Qatar National Outbreak Control Task Force (OCT) to find answers for the novel disease to control the outbreak and inform the public. During the early phase of the MERS-CoV epidemic, the OCT had to decide from where to start and which methods to follow.

The One-Health approach focuses on the association and interconnection between three sectors: human, animal and environmental health, and recognizes the total health and wellbeing of these three sectors [5]. One health is defined as “A collaborative, multisectoral, and transdisciplinary approach - working at the local, regional, national, and global levels - with the goal of achieving optimal health outcomes recognizing the interconnection between people, animals, plants, and their shared environment” [6]. This view point describes how the One-Health approach was used in surveillance and response to MERS-CoV in Qatar during the period 2012 to 2017. It could be useful to similar authorities to inform their preparedness plans for the next potential emerging zoonotic epidemic in light of the strengths and challenges experienced by Qatar.

ACTION TAKEN

The OCT was reactivated in Qatar in November 2012 following the detection of the second MERS-CoV case (sixth worldwide) in the country. The OCT was established before as a requirement of the International Health Regulation (IHR) (2005). It is a joint outbreak investigation team composed of public and animal health experts lead by the Ministry of Public Health (MoPH) in Qatar, applying the One-Health approach and dealing with zoonotic infections. The OCT played a fundamental role in the national response to the Severe Acute Respiratory Syndrome (SARS) and Influenza (H1N1) virus pandemic in 2009. The new OCT involved multidisciplinary and multisectoral representation primarily from the MoPH and the Ministry of Municipality and Environment (MME) in Qatar. To develop a coherent investigation and response strategy, the MoPH leaders decided to support the OCT with technical expertise from the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO), the Centres for Disease Control and Prevention of the United States (CDC), Public Health England (PHE), and Erasmus Medical Centre (EMC) in the Netherlands.

Experts from these institutions engaged the OCT in an extensive risk assessment of the MERS outbreak situation. A comprehensive road map was developed to provide guidance on surveillance, investigation and response, using a joint One-Health approach. This road map included: (1) Coordination and joint leadership, (2) Joint surveillance and field investigation, (3) Epidemiological studies, and (4) Increase of local diagnostic capacity.

Coordination and joint leadership: The road map emphasized the fostering of a conjoined leadership in terms of planning and decision making. An improved level of coordination was witnessed thereafter between authorities across a number of areas; sharing of information turned into timely practice, the process for decision making and approval of plans became quicker, access to allocated resources and high risk areas (e.g., slaughter house, animal holdings, camel racing areas, hospitals etc.) became easier, and communicating the risk to the public reflected one voice for joint authorities.

Joint surveillance and field investigation: A joint Rapid Response Team (RRT) was assigned from the MoPH and the MME to carry out field investigation after being subjected to refresher training on skills and principles of outbreak investigation. To initiate One-Health surveillance it was agreed to build the RRT on the already functioning Severe Acute Respiratory Infections (SARI) surveillance team in the Public Health Department. The SARI team routinely searches for SARS and Influenza virus among suspected cases admitted to hospitals with respiratory symptoms. Importantly, the community was also engaged in this form of surveillance as family members, friends and co-workers of the suspected cases were educated about MERS symptoms and how to report them

The ability to access and investigate all of the 24 reported cases within 24-48 hours of reporting was one of several fruits of the One-Health surveillance, permitting to yield further epidemiological evidence on the potential role camels play in the virus transmission.

Whenever a human case was suspected with MERS infection, a patient investigation was conducted, which included contact tracing, work and movement of the patient and history of camel contact. If the patient had direct or indirect camel contact, the RRT informed their veterinary counterpart. The veterinary team tested the camels around the patient. The public health team tested all human contacts around the patient. Later on, a complete report of that patient was prepared. Based on these reports, a complete One-Health approach was developed and implemented. At least 3 MERS cases were detected during routine case investigations and contact tracing at the community settings.

Epidemiological studies: The One-Health road map recommended to start with a case-control study and seroepidemiological surveys targeting at-risk populations including humans and camels besides testing the stored human respiratory samples retrospectively to determine whether the virus was totally novel to Qatar population. The outcome of joining forces in carrying out these studies was outstanding. First and foremost it helped facilitate the access of the joint RRT to the camel barns and the farms hosting race camels. Collection of samples and data of camels and their caregivers became easier, allowing a series of subsequent fundamental epidemiological, veterinary and clinical studies to take place.

Through these studies it was possible to provide the first global molecular evidence that camels are a potential source for MERS-CoV [4]. The door was opened to a number of research studies that ensued thereafter to yield insights into some essential aspects of MERS-CoV. While some studies focused on exposure to camels and the husbandry practices, others addressed the mode of transmission to humans, and possible risk factors [4, 7-14]. These findings have been used to provide further guidance for studies and prevention measures in Qatar and other affected countries.

Increase the local diagnostic capacity: During the early days of the outbreak, the Influenza laboratory in the National Influenza Centre of Hamad Medical Corporation, Qatar was the primary destination to examine MERS-CoV samples. As the epidemic evolved and the OCT reactivated, the workload to diagnose human or camel specimens gradually increased. Joint training activities for both human and animal labs were organized on lab detection of MERS-CoV. MERS-CoV sample storage and the test capacity in both human and veterinary laboratories were scaled up. A network including international reference laboratories was initiated and this alliance is maintained to date to tackle other zoonotic diseases like Influenza, Rabies etc. To improve coordination and communication, a focal person was assigned from human and veterinary laboratories to ensure timely data and sample sharing.



FACILITATORS AND BARRIERS

The commitment of the MoPH and MME in Qatar to the One-Health approach that governed the response to MERS-CoV was largely due to the previous experiences with SARS and H1N1 pandemic as these experiences helped to show the feasibility and advantages of joint efforts to policy-makers. The joint strategic technical collaboration between the Qatari authorities and the international technical bodies such as the WHO, FAO, CDC, PHE and EMC have greatly facilitated the preparation and implementation of the road map. Community engagement was another key factor that improved the investigation of suspected cases.

Yet, these achievements were not without obstacles. As camels infected with MERS only show asymptomatic or mild respiratory tract disease, camel owners and workers doubted the link between camels and the disease. Moreover, the veterinarians showed insufficient interest in mounting a large scale outbreak operation similar to the one initiated by the public health sector, as they have a shortage of technical guidance on surveillance and other technical areas including laboratory tools and diagnostic kits. The One-Health approach needed more local and international support to ensure a systematic and sustained implementation. Furthermore, one study on risk perception suggested that some camel owners have poor risk perception of MERS [15]. Additionally, both public health and veterinary sectors were accustomed to work in a solo vertical way and only occasionally engaged with each other in short term research projects. Apart from the SARS and H1N1 epidemics, the two sectors worked independently, making joint technical work a difficult task. Therefore, the livestock sector did not appear to feel the same pressure as the public health sector. However, further studies are required to explore areas for improvement to ultimately make the One-Health approach more appealing to all sectors involved.

LESSONS LEARNT FROM THE FIELD

The One-Health approach, despite lacking the appropriate technical guidance, was already functional and helped address some zoonotic diseases including Influenza and Brucella. Although capabilities and funding were unequal between the public health and the veterinary sector, the available competencies, supported with a substantial political will to join efforts and improve coordination, were sufficient to jointly address MERS-CoV. The adopted inter-sectorial collaboration for surveillance has been vital to obtain a better understanding of MERS-CoV in Qatar. Obtaining the reliable evidence about transmission between camels and humans could have never been achieved without the prompt and timely joint investigation. Building local One-Health technical capacity to investigate and confirm MERS-CoV in humans and animals helped the early detection of cases in humans and animals. Such practices minimized the time and costs for public health control measures.

Community engagement has been key to establishing One-Health surveillance in Qatar. Self-reporting of disease compared with the previous rejection and denial was an important change of behaviour among the people at risk, particularly those exposed to abattoir, camel race areas and the ports of entry. Furthermore, the positive community response to the MERS-CoV outbreak control policy was due to the constant transparent emergency risk communication which allowed the community to be well-informed of the situation. It also helped to maintain public trust in the competency of national authorities.

The One-Health approach has been essential for generating evidence and implementing control measures to restrain MERS-CoV and other zoonotic diseases. The same approach needs to be maintained to assess the effectiveness of the control measures. As emerging zoonotic viruses continue to be a challenge, One-Health surveillance must be adopted and fostered at all levels [5].

Finally, as the human MERS-cases seemed to uniquely emerge from the Arabian Peninsula, regional collaboration in sharing clinical and surveillance data besides the results of scientific research is indispensable to finding answers to the remaining gaps in our knowledge about the disease. The unexpected disruption of the GCC countries undoubtedly hindered an effective regional collaboration in sharing data and carrying out sequencing studies. International agencies are required to call upon them to consider the One-Health approach building on the Qatar experience which displayed a practical way of sharing resources and avoiding obstacles to work in the Arabian community. Establishing a regional committee for coordination and emergency risk communication is an important element to build capacities required for zoonosis control. We suggest that a regional 'One-Health Centre of Excellence (OCE)' would help to develop unified standards and integrative guidelines for control of zoonoses including MERS-CoV.

Conflict of interest

Authors declare no conflict of interest with regard to their views concerning the implementation of the One-Health approach in Qatar.

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Chapter 7

Survey on implementation of One Health approach for MERS-CoV preparedness and control in Gulf Cooperation Council and Middle East countries

FARAG, E. A. B., NOUR, M., EL IDRISI, A., BERRADA, J., MOUSTAFA, A., MEHMOOD, M., MAHMOUD, M. H., EL-SAYED, A. M., ALHAJRI, F., AL-HAJRI, M., HASSAN, O. A., AL-ROMAIHI, H., AL-THANI, M., AL-MARRI, S. A., KOOPMANS, M. P. G. & ISMAIL, M. H. 2019c. Survey on Implementation of One Health Approach for MERS-CoV Preparedness and Control in Gulf Cooperation Council and Middle East Countries. *Emerg Infect Dis*, 25.

ABSTRACT

In 2015, a One Health Working Group was established in Qatar to conduct a survey in the Gulf Cooperation Council countries, Egypt, and Jordan to monitor preparedness of public health and veterinary health authorities in response to the Middle East respiratory syndrome coronavirus epidemic. All but 1 country indicated they established joint One Health policy teams for investigation and response. However, the response to the questionnaires was largely limited to veterinary authorities. Critical barriers and limitations were identified. National and regional leaders, policy makers, and stakeholders should be prompted to advocate and enhance adoption of the One Health framework to mitigate the risk for Middle East respiratory syndrome and other emerging zoonotic diseases.

BACKGROUND

Human infections with Middle East respiratory syndrome coronavirus (MERS-CoV) continue to be reported from the Arabian Peninsula and the Middle East after the September 2012 World Health Organization (WHO) notification of 640 deaths from 2,040 laboratory-confirmed cases (1). Although typical symptoms of MERS-CoV infection include fever, cough, and labored breathing, pneumonia and diarrhea also were reported. Asymptomatic persons with laboratory-confirmed cases were observed as well (2). Saudi Arabia, the first country to report a confirmed MERS-CoV case, has had the most reported cases. Studies in Qatar and Saudi Arabia established the link between MERS-CoV and dromedary camels (1). Camels are valued animals in arid and semiarid regions (3), where they serve as a basic source of milk and meat (4). The trading of camels and camel meat is an important source of income (5). In addition to the use of camels for food production, camels are popular for sport competition and beauty championships, which has led to formation of special camel institutions in some Arabian countries, including camel supreme councils and camel hospitals.

With the MERS-CoV outbreak as an emerging threat, the public health response included the possible role of camels in collaborative work with veterinary authorities to control and prevent the disease. Uncertainties about MERS-CoV transmission modes, coupled with growing evidence of the potential role of camels in disease dissemination, made this first trial of a One Health response challenging. A proper One Health response to a zoonotic disease requires several elements, including political support, appreciable preparedness and response plans, a joint vision on epidemiologic surveillance for MERS-CoV and zoonotic diseases in general, joint use of laboratory diagnostic capabilities, funding, and means for crisis communication and health education.

In Qatar, led by the Supreme Council of Health, a multidisciplinary team was established in 2014 once the zoonotic origin of the disease became evident. To discuss the challenges encountered during the MERS-CoV outbreak, and as part of international efforts to advance the adoption of the One Health approach to address health risks at the animal–human–environment interfaces (6), together with the Food and Agriculture Organization of the United Nations (FAO), Qatar organized in April 2015 a regional workshop in collaboration with the World Organisation for Animal Health (OIE) and WHO about the application of the One Health approach to MERS-CoV (7). Countries from the Gulf Cooperation Council (GCC; Saudi Arabia, Qatar, Kuwait, United Arab Emirates, Bahrain, and Oman), Egypt, and Jordan were represented in this workshop, along with delegates from FAO, OIE, WHO, the US Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA), Erasmus Medical Center (Rotterdam, the Netherlands), the University of Hong Kong, and several other international experts.

To gauge a preliminary understanding about the extent to which the involved countries were using a One Health approach and how it was translated in government policies and

Table 1: Domains of the questionnaire of the survey on the implementation of One Health for MERS-CoV preparedness and control in Gulf Cooperation Council and Middle East countries, 2015*

Principle	Title
1	Leadership and Coordination
2	Policies and Drivers of MERS-CoV Management
3	Preparedness and Response Plans
4	Epidemiologic Surveillance System
5	Laboratory Diagnostic Capacities
6	Crisis Communication and Health Education
7	The One Health Approach Operationalization Challenges

*MERS-CoV, Middle East respiratory syndrome coronavirus.

practices, the One Health Working Group conducted a survey before the workshop. The findings were aggregated, presented, and discussed before the entire audience of the workshop.

METHODS

We designed the study based on guidance and references of the One Health approach established in documents issued by FAO, OIE, WHO (6), and CDC (8); meeting reports (9,10); and policy documentation (11). A questionnaire was drafted to answer queries about policies and structures governing control of zoonotic diseases in general and MERS-CoV in particular (Table 1). We shared the questionnaire with public health and veterinary authorities in charge of surveillance and control of MERS-CoV in all GCC countries, Egypt, and Jordan 1 week before the workshop. The questionnaire also included open-ended questions permitting comments. Results were analyzed and interpreted using an Excel spreadsheet (Microsoft, <https://www.microsoft.com> External Link). The core of the questions and the relevant results scores are shown in Table 2. Results were presented and discussed before the survey participants and audience of the workshop and approved by the joint scientific committee of the workshop. Decision for dissemination followed consent of all of the survey participants.

RESULTS

We surveyed 16 authorized government institutions representing 8 countries. Two countries did not respond. Seven (43%) institutions from 6 (75%) countries responded to the questionnaire. Six (85%) of 7 responding institutions were veterinary authorities. Except for 1 country, no public health authorities responded to the questionnaire.

Table 2: Outcomes of survey questionnaire on the implementation of One Health for MERS-CoV preparedness and control in Gulf Cooperation Council and Middle East countries, 2015*

Domain	Subdomain	Response	
		Yes	No
Leadership and coordination	A. Existence of a dedicated MERS-CoV committee in surveyed institutions†	7	0
	B. The committee is meeting on regular basis†	6	1
	C. Participation of stakeholders in a joint committee or advisory board dealing with MERS-CoV at the national level†	5	2
	D. Activation of emergency supreme committee for MERS-CoV at the state level‡	2	4
Policies and drivers of MERS-CoV management	A. Existence of a document ascribing policy, roles, and responsibilities of committee's stakeholders†	5	2
	B. The document describes the chain of command‡	4	2
	C. Joint committee responsibility for preparedness and response to MERS-CoV†	5	2
Preparedness and response plans	A. National plans for preparedness and response to MERS-CoV†	6	1
	B. Participation of stakeholders in preparation of national plans for preparedness and response to MERS-CoV‡	2	4
	C. Adequate budget allocation†	2	3
Epidemiologic surveillance system of MERS-CoV	A. Program of epidemiologic surveillance in humans‡	6	0
	B. Program of epidemiologic surveillance in animals‡	5	1
	C. Participation of animal breeders in MERS-CoV epidemiologic surveillance‡	3	3
	D. Joint or integrated surveillance program for MERS-CoV†	4	2
	E. MERS joint field investigation team†	5	2
	F. Field investigation joint team training‡	2	2
	G. Research program(s) for MERS-CoV†	2	4
Laboratory diagnostic capacities‡	A. Public Health Reference Laboratory	4	2
	B. Veterinary Reference Laboratory		
Crisis communication and Health education	A. Strategies and plans for information, crisis communication, and health education on MERS-CoV†	5	2
	B. MERS-CoV communication cooperation and coordination‡	5	1
	C. Joint implementation of MERS-CoV awareness and health education activities‡	3	3

*MERS-CoV, Middle East respiratory syndrome coronavirus.

†Statistical analysis was performed by institution.

‡Statistical analysis was performed by country.

Leadership and Coordination

The 6 responding countries reported the existence of a joint veterinary and public health MERS-CoV committee (Table 2). Six institutions confirmed meeting on a regular basis. Five institutions from 4 countries reported having joint committees encompassing public health, veterinary services, municipalities, and research authorities. Two countries had an active emergency supreme committee at the national level addressing MERS-CoV crisis and threat.

Policies and Drivers of MERS-CoV Management

Five institutions from 4 countries reported the presence of national documents detailing entitled authorities, policies, roles, commands, and responsibilities for stakeholders involved in MERS-CoV management. The same 5 institutions reported having a joint public health–veterinary authority committee responsible for preparedness and response to MERS-CoV following the standardized procedures developed by FAO and WHO.

Preparedness and Response Plans

Six institutions from 5 countries had national early preparedness and response to MERS-CoV plans. Four of these institutions had clearly defined roles and responsibilities for each of the involved authorities (public health authority, animal health authority, environment authority, and others) during MERS-CoV threat or outbreaks. Only 2 (33%) countries had involved the major stakeholders (public health and animal health authorities) in the process of preparing a national plan for preparedness and response to MERS-CoV. Of the 7 institutions that answered the questionnaire, 2 reported adequate funding to address MERS-CoV, 3 denied adequacy, and the remaining 2 did not respond. Two institutions from 1 country did not agree on funding questions.

Joint Epidemiologic Surveillance of Zoonotic Pathogens

All but 1 country reported having established a MERS-CoV epidemiologic surveillance program investigating vulnerable animals, camel owners, camel workers, breeders and keepers, slaughterhouse workers, and veterinary and medical personnel and sharing data with counterparts. Three countries reported participation of animal breeders; the other 3 reported the contrary. Four institutions from 4 GCC countries reported the existence of a joint epidemiologic surveillance program enabling outbreak investigation and sharing of reports and results. Two institutions reported lacking the joint surveillance, and 1 did not respond.

In 4 countries, 5 of 7 institutions indicated the presence of a joint public health–veterinary authority field investigation team and that MERS-CoV was jointly investigated. Two of the 4 countries organized an epidemiologic and disease control training course for the joint investigation team. Two countries initiated research programs in response to the outbreak.

Joint Laboratory Diagnostic Capabilities

In 4 countries, national reference laboratories were established and identified to provide diagnostic services for human and animal MERS-CoV infection. The 4 countries reported national collaboration encompassing laboratory services, joint MERS-CoV diagnosis training, specimen shipping, and competency testing. Regionally, 2 GCC countries reported joint laboratory processing for MERS-CoV in camel samples. Three GCC countries reported joint activities with the Netherlands, Hong Kong, Germany, CDC, and the UK reference laboratories to fulfill international diagnostic and research requirements.

Crisis Communication and Health Education

Five of 7 responding institutions from 4 countries reported having MERS-CoV crisis communication and health education strategic plans stating that the key stakeholders were involved in plans development. The 2 remaining countries either did not include these strategies in their national plans or were not aware of inclusion of these strategies.

Six responding countries reported providing MERS-CoV communication coordination mechanisms between public health and veterinary authorities covering awareness and health education. One country reported some conflicting messages between the 2 authorities. Three of the responding countries reported collaboration and implementation of awareness and health education issues during the MERS-CoV epidemic. In all but 1 country, camel breeders did not participate in the campaign.

The One Health Approach Operationalization Challenges

Four of the 6 responding countries reported operational challenges encountered with adoption of the One Health approach. These challenges included lack of reliable and specialized diagnostic laboratories in the region, incapacity of the existing laboratories to yield MERS-CoV diagnostic services, and lack of skilled personnel tasked to investigate zoonotic cases. Other reported key challenges were misunderstanding of the One Health concept; conflicting priorities and plans; dearth of budgets allocated to meet MERS-CoV technical needs in terms of surveillance, diagnosis, control, and research; lack of skilled personnel on communication and health education; and the denial of camel breeders.

DISCUSSION

Because of the global increase in zoonotic threats, the importance of the One Health approach has also increased, along with the need to establish effective mechanisms for collaboration to address threats at the human–animal–environment interface (6,8,12,13). Affected by the United Nations agencies, several countries, particularly those challenged by zoonotic events, began initiating their One Health platforms and programs to enhance their capacities to manage zoonotic diseases (10,11,14–16). However, these efforts always faced many challenges.

To enable sufficient internal deliberations and ensure One Health quality and consensus-based responses, we shared the survey questionnaire with the relevant authorized health and veterinary institutions. However, the first hindrance was the response by only 44% of surveyed institutions, a fact that limited a comprehensive analysis of the outcomes. This low response rate could be attributed to poor leadership and to limited conceptual awareness about the One Health approach (16,17). This finding is sustained by our observation that there was a discrepancy understanding the One Health approach. Although the term is familiar among veterinarians, it is not among their health counterparts, a considerable

drawback to implementing the approach. A high-capacity endeavor is needed advocating the health sector to deal with the One Health approach in the future.

Most of the GCC countries, including those with high MERS-CoV incidence, have adopted an epidemic control policy, indicating that the One Health approach was either partially embraced or totally overlooked. This finding was demonstrated by the fact that only 2 responding countries reported veterinary health authorities partnership formulating national preparedness and response plans. As a result, the quality of data collected in response to an outbreak remains questionable.

The lack of budget to support MERS-CoV control programs revealed by the survey questionnaire and the consequent workshop discussions emphasizes crucial points in the implementation of a successful One Health approach. One explanation may be that the cost for a proper One Health response had been underestimated. However, the disproportionate distribution of the available budget raised by the delegates might further explain the lack of integrated response. For instance, although most surveyed countries had established MERS-CoV epidemiologic investigation teams, only 50% of these teams react jointly. At the level of diagnostics, national laboratories in 66% of the countries managing and diagnosing MERS-CoV outbreaks had collaboration between medical and veterinary response, and several teamed up with international reference laboratories, which was considered a positive step toward diagnostic efficiency and cooperation. However, because MERS-CoV is a GCC home-country infection, the in-country diagnostic capacity was expected to be adequate.

When discussing crisis communication and health education, the core persistent barrier to embracing One Health seemed to be the prevalent denial of the camel owners that camels could be a potential source of MERS-CoV. Because of the highly influential role of camel owners among the communal sectors of most of the surveyed countries, involvement of these sectors to combat emerging zoonotic diseases is essential (18,19). However, because most local communities tend to react forcibly toward emerging infectious diseases (20), the investigators could neither judge this factor nor its effect in curbing the policy makers bolstering the One Health approach (21). Anticipating such socioeconomic risk factors, involvement of social scientists to resolve this barrier might help (22) facilitate community buy-in of One Health.

The survey results appear to show that respondents did not benefit much from the lessons learned during the last influenza A(H1N1) outbreak (23). The variation in the nature of MERS-CoV epidemiology among the countries—handled as a human-associated infection in some, a human–camel infection in others, and an unnoticed inapparent camel infection in others (2)—has imbalanced the magnitude of response among healthcare and veterinary sector authorities, a situation negatively affecting the application goals.

Given that the One Health approach is increasingly recognized internationally as an effective trend for managing emerging diseases at the human–animal–environment interface (10,11,18), the key barrier fostering the One Health approach at the national level

suggested by this study seems to be the relative lack of political will. Based on the experience gained in addressing MERS-CoV at the human–animal interface, this lack of will could further be responsible for the poor sectoral response to the surveillance questionnaire. Although in Qatar, MERS-CoV was addressed through a One Health approach from the start (24), much remains to be done nationally, particularly at policy-making level. The foundation of a permanent interministerial committee might be a key step to raise awareness of leaders and policy makers using the concept and to determine the importance of the One Health approach. Creation of a supreme coordinating crisis communication committee is an important element to build zoonosis control and prevention capacities. A unified funding policy is a good incentive encouraging alleviation of the financial obligations accompanying One Health, expected to ease launching of joint investigations, intensive health educational sessions, epidemiologic surveillance programs, and joint seminars and workshops. Sharing of laboratory diagnostic research facilities, diagnostic protocols, and application of proficiency testing would help build experience and improve quality results. Joint routine veterinary health services programs application and adoption of compensation policy with continuous health education and extension programs might turn animal owners and other social stakeholders onto One Health.

The ratification of establishing a regional GCC center for infection control (25) to help develop unified standard and integrative guidelines to control zoonoses might help sustain the One Health approach. However, whether the current political situation might compromise the hope created by the previously promised political commitment to collaborate and allocate funds after the recent emergence of avian influenza A(H5N1) (26) remains questionable.

Dr. Farag is the Acting Head of Communicable Diseases Control Programs, Public Health Program, Ministry of Public Health, Doha, Qatar, His primary research interest is emerging infectious diseases.

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Chapter 8

Global status of Middle East respiratory syndrome coronavirus in dromedary camels: a systematic review

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ABSTRACT

Dromedary camels have been shown to be the main reservoir for human Middle East respiratory syndrome (MERS) infections. This systematic review aims to compile and analyse all published data on MERS-coronavirus (CoV) in the global camel population to provide an overview of current knowledge on the distribution, spread and risk factors of infections in dromedary camels. We included original research articles containing laboratory evidence of MERS-CoV infections in dromedary camels in the field from 2013 to April 2018. In general, camels only show minor clinical signs of disease after being infected with MERS-CoV. Serological evidence of MERS-CoV in camels has been found in 20 countries, with molecular evidence for virus circulation in 13 countries. The seroprevalence of MERS-CoV antibodies increases with age in camels, while the prevalence of viral shedding as determined by MERS-CoV RNA detection in nasal swabs decreases. In several studies, camels that were sampled at animal markets or quarantine facilities were seropositive more often than camels at farms as well as imported camels vs. locally bred camels. Some studies show a relatively higher seroprevalence and viral detection during the cooler winter months. Knowledge of the animal reservoir of MERS-CoV is essential to develop intervention and control measures to prevent human infections.

INTRODUCTION

Middle East respiratory syndrome (MERS) is a highly fatal respiratory tract disease in humans that was first detected in 2012 in the Kingdom of Saudi Arabia (KSA) [1]. After its first detection, MERS-coronavirus (MERS-CoV) was being reported in human patients across the Arabian Peninsula, with occasional travel-related cases in other continents. As of the end of March 2018, a total of 2189 human laboratory-confirmed cases from 27 countries have been reported to the World Health Organisation (WHO), including 782 associated deaths [2]. Dromedary camels (*Camelus dromedaries*) have been shown to be the natural reservoir from where spill-over to humans can occur [3, 4]. Human-to-human infection is also reported frequently, especially in healthcare settings [5]. Sustained human-to-human transmission outside of hospital settings has not been shown yet [6]. Direct or indirect human contact with camels has resulted in repeated introductions of MERS-CoV into the human population [7]. It has been suggested that camels may have acquired MERS-CoV from a spill-over event from a bat reservoir, but evidence for that remains inconclusive [8]. Infections with MERS-CoV generally are thought to be mild or inapparent in camels [9], and are therefore of low economical or animal welfare significance. This systematic review was done to compile and analyse all published data on MERS-CoV in the global camel population to provide an overview of current knowledge on the distribution, spread and risk factors of MERS-CoV infections in dromedary camels as a basis for the design of intervention and control measures to prevent human infections.

This systematic review was done to compile and analyse all published data on MERS-CoV in the global camel population to provide an overview of current knowledge on the distribution, spread and risk factors of MERS-CoV infections in dromedary camels as a basis for the design of intervention and control measures to prevent human infections.

MATERIAL AND METHODS

On 2 May 2018, a literature search on PubMed was performed, using the terms ‘middle east respiratory syndrome coronavirus’ and ‘MERS-CoV’. Using the term ‘MERS’ did not result in any additional articles that fit the scope of this review. Only articles published in English were included. Two reviewers individually selected all original research articles containing laboratory evidence of MERS-CoV infections in dromedary camels in the field. Articles that were mentioned in Food and Agriculture Organization (FAO) updates [10] or in the references of included publications, but did not appear in the PubMed search were added. Subsequently, abstracts, follow-up studies of MERS-CoV-positive camels and genome studies without prevalence data were excluded from the analysis. Data on variables such as year of sampling, country, region, age, sex and animal origin were extracted and analysed. For each variable, the number of positive camels, total number of camels tested and the

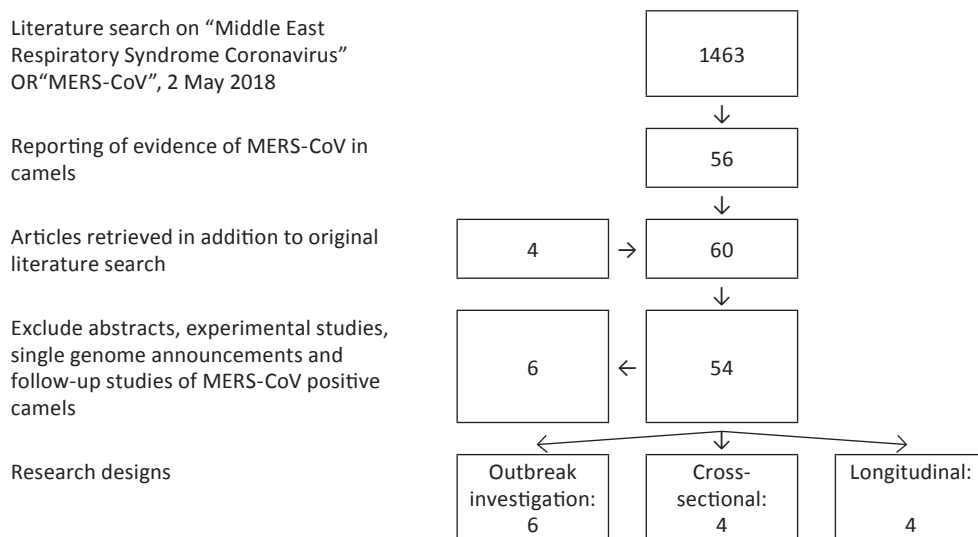


Figure 1: results literature search

median percentage positivity was calculated. Data from experimental infection studies were not included in this analysis, but they were included in the review to provide additional information and context to the field studies. Additional information on the distribution and trade of dromedary camels was collected from references in the publications on MERS-CoV in camels and extracted from official FAO and World Organisation for Animal Health (OIE) databases [11, 12]. The additional literature on camel trade was collected in a less systematic way from PubMed.

RESULTS

Literature search

The literature search resulted in a total of 53 papers (Fig. 1). Forty-three research papers described the results of cross-sectional studies in dromedary camel populations, six papers described outbreak investigations, including an analysis of camel samples, and four papers described longitudinal studies. In total, 33 papers describe camel studies in the Middle East, 13 studies investigated camels from Africa and the remaining seven surveys were from Spain, Australia, Japan, Bangladesh and Pakistan (Table 1).

Distribution and trade of camels

Most recent FAO statistics estimate the world population of camel to be around 29 million [11], of which approximately 95% are dromedary camels [13]. However, it is believed that the true population size is even larger due to inaccurate statistics and feral camels, such as

the feral dromedary camel population in Australia that is estimated to be around 1 million [14]. Over 80% of the camel population lives in Africa. The main camel countries are Chad (6 400 000), Ethiopia (1 200 000), Kenya (2 986 057), Mali (1 028 700), Mauritania (1 379 417), Niger (1 698 110), Sudan (4 830 000), Somalia (7 100 000) and Pakistan (1 000 000) [12] (Table 2). A large number of camels are being transported from the Horn of Africa to the Middle East each year. These are mainly meat camels coming from the east of Africa going to Egypt, Libya and the Gulf states, and Sudanese camels that are being imported into the Middle East to participate in camel racing competitions [15]. For example, the FAO reported that Somalia exported 77 000 camels in 2014 [16]. The largest camel market in Africa is the Birqash market near Cairo (Egypt), where camels from Sudan and Ethiopia are most common, but trade routes include animals from Chad, Somalia, Eritrea and Kenya [17]. Imported camels are usually quarantined for 2–3 days at the border before they are allowed to enter Egypt [17]. Most Somali and Sudanese camels that are exported to the KSA are shipped from the ports of Berbera and Bosaso in North Somalia to the KSA ports of Jizan and Jeddah [15].

Clinical and pathological features of MERS-CoV infections in dromedary camels

In general, only minor clinical signs of disease have been observed in animals infected with MERS-CoV and most MERS-CoV infections do not appear to cause any symptoms [9]. Disease symptoms that have been described after experimental and field infections are coughing and sneezing, respiratory discharge, fever and loss of appetite [18–20]. Although MERS-CoV RNA can be detected in several organs after experimental infection, in studies of natural infectious virus it has only been detected in the tissues of the upper and lower respiratory tract and regional lymph nodes of the respiratory system in part of the infected camels. Histologically, a mild-to-moderate inflammation and necrosis could also be seen on the upper and lower respiratory tract. No viral antigen or lesions were detected in the alveoli. Histopathological examination showed that the nasal respiratory epithelium is the principal site of MERS-CoV replication in camels [18, 21].

Virus shedding and antibody response

In one study investigating experimental infection of camels, MERS-CoV shedding started 1–2 days post-infection (dpi). In that study, infectious virus could be detected until 7 dpi, and viral RNA until 35 dpi in nasal swab samples and, in lower amounts, in oral swab samples [18]. No infectious virus or viral RNA was detected in faecal or urine samples [18]. Viral RNA detection in nasal, but also rectal swabs of camels after experimental infection until day 14, has been confirmed in a recent vaccine study [21]. In the field surveys included in this review, MERS-CoV RNA has been described in rectal swab samples, although other field studies report negative results [3, 22–24] and when viral RNA can be detected, the positivity rate of rectal swabs is lower compared with nasal swab samples [19, 25–27]. Oral swabs are usually negative or show a lower positivity rate even when nasal swabs test

positive for MERS-CoV RNA [3, 19, 26]. Some studies have reported MERS-CoV RNA in milk samples [27, 28]. Longitudinal studies of camel herds show that PCR results of nasal swabs can remain positive after 2 weeks [27, 29]. When an interval of sampling of 1 or 2 months was maintained, nasal swabs become negative for viral RNA in the next sampling round [24, 30]. MERS-CoV infections have also been detected in camels with MERS-CoV antibodies, both in calves with maternal antibodies as well as older camels that had already acquired antibodies from a previous infection. However, virus replication and thus the virus load is generally lower in infected seropositive animals compared with seronegative camels [19, 21, 23, 24, 30, 31]. Little is known about the longevity of antibody titres after infection from longitudinal studies. A study following camels on a closed farm found that neutralizing antibodies remained consistent during a year [30], while other studies found that antibody titres rapidly drop by 1–4-fold within a period often as short as 2 weeks [24, 27].

Worldwide distribution of MERS-CoV in dromedary camels

The first evidence of MERS-CoV in camels described so far is the detection of antibodies to MERS-CoV in camel sera from Somalia and Sudan from 1983 of which 81% tested positive [32]. Additional serological evidence of the widespread presence of MERS-CoV infection in camels, included in this review, has been found in 18 additional countries: Bangladesh, Burkina Faso, Egypt, Ethiopia, Iraq, Israel, Jordan, Kenya, KSA, Mali, Morocco, Nigeria, Oman, Pakistan, Qatar, Spain, Tunisia and the UAE (Fig. 2). In addition, Promed mail reported that virus-positive camels had been found in Kuwait and Iran, the latter reportedly in imported animals (Archive number 20140612.2534919 and 20141029.2912385). In 11 countries, serological findings were complemented with the finding of viral RNA in dromedary camels: Burkina Faso, Egypt, Ethiopia, Iraq, Jordan, KSA, Morocco, Nigeria, Oman, Qatar and the UAE. Investigations of MERS-CoV circulation amongst dromedary camels in Australia, Japan, Kazakhstan, USA and Canada did not find any proof of MERS-CoV circulation. All countries where MERS-CoV circulates in the camel population, with the exception of Spain (Canary Islands), Pakistan and Bangladesh, are located in the Middle East or Africa [4, 33]. One out of 17 camels that had MERS-CoV antibodies in Bangladesh was born in Bangladesh, 16 others were imported from India [34]. However, there have not been any additional reports of MERS-CoV in camels in India. There is no record of foreign origin of the seropositive camels from Pakistan [35]. Moreover, in previous studies there had already been evidence of seropositive camels that originate from Pakistan [37, 58].

When combining serology data from all papers included in this review, the overall median seroprevalence of camels in Africa is 81% (6106/8526; range 28–98%), compared with a median seroprevalence of 93% (3230/3846; range 53–100%) in camels from the Middle East. Based on viral shedding studies from African countries, the median rate of viral shedding was 5% (1108/6318; range 1–15%), compared with 12% in camels from the Middle East (1191/14902; range 0–100%).

Risk factors of MERS-CoV in dromedary camels

Table 1

References	Study design	Country of origin	Year	MERS-CoV RNA presence	MERS-CoV sero-prevalence	Sex	Age	Imported/ local	Sampling location	Other animals tested
Hemida et al. [50]	Cross-sectional	KSA	2010-2013	pp: 90% (280/310)			<1Y: 72% (47/65) 1-3Y: 95% (101/106) 4-5Y: 97% (74/76) >5Y: 92% (58/63)			Sheep 0% (0/100) Goat 0% (0/45) Chicken 0% (0/240) Cattle 0% (0/50)
Perera et al. [48]	Cross-sectional	Egypt	2013	MN: 98% (108/110)					Abattoir	Goat 0% (0/13) Sheep 0% (0/5) Buffalo 0% (0/8) Cattle 0% (0/25) Swine 0% (0/260) Wild birds (Hong Kong) 0% (0/204)
Reusken et al. [4]	Cross-sectional	Oman	2013	Protein Microarray (pMA): 100% (50/50) 14% (15/105)	Female: 100% (50/50) Male: 4% (2/50) Female: 13% (7/55)		8-12Y: 100% (50/50)	Local	Breeding farm	Bactrian camel 0% (0/4) Alpaca 0% (0/24) Llama 0% (0/7) Guanaco 0% (0/2) Cattle 0% (0/40) Goat 0% (0/120) Sheep 0% (0/40)
Reusken et al. [51]	Cross-sectional	Spain (Canary islands) Jordan	2012-2013 2013	Fecal: 0% (0/11) pMA: 100% (11/11)	Male: 100% (11/11)		3-14m: 100% (11/11)	Morocco: 0% (0/3)	Tourist farm	Sheep: 0% PCR (0/126) pMA: 5% (6/126) : 0% (0/126) Cattle: PCR 0% (0/91) pMA: 0% (0/91) Goat: pMA/ 0% (0/150) Goat: PCR 0% (0/36) ELISA 0% (0/35) Sheep: PCR 0% (0/78) ELISA 0% (0/112)
Alagaili et al. [31]	Cross-sectional	KSA	1992-1993 1993-1994 1996 2004 2009 2010 2013	ELISA: 100% (1/1) 100% (2/2) 93% (114/123) 100% (6/6) 100% (6/6) 78% (64/82) 84% (37/44) 74% (150/203)		<2Y: 52% (50/96) 2-5Y: 88% (29/33) >5Y: 98% (54/55)				
Alexandersen et al. [49]	Cross-sectional	UAE	2005	82% (9/11)	Male: 50% (2/4) Female: 100% (7/7)					Sheep 0% (0/20) Horse 0% (0/3)
		USA & Canada	2000-2001	0% (0/6)						

Table 1: Continued

References	Study design	Country of origin	Year	MERS-CoV RNA presence	MERS-CoV sero-prevalence	Sex	Age	Imported/local	Sampling location	Other animals tested
Azhar et al. [66]	Human outbreak investigation	KSA	2013	Nasal: 11% (1/9) Milk, urine, rectal: 0% (0/11)	IFA/ELISA: 100% (9/9)		<1Y: PCR 33% (1/3) IFA/ELISA: 100% (3/3) 2-5Y: IFA/ELISA 100% (1/1) >5Y: IFA/ELISA 100% (5/5)	Farm	Farm	
Memish et al. [67]										
Chu et al. [9]	(Multiple) cross-sectional	Egypt	2014	Nasal: 4% (4/93)	pp: 92% (48/52)		>6Y: 92% (48/52)	Sudan or Ethiopia	Abattoir	-
Corman et al. [36]	Cross-sectional	Kenya	Total	Nasal: 0% (0/17)	ELISA, total: 30% (228/774)		Adult: 37% (226/70) Juvenile: 25% (15/59)	Local	Farm	-
			1992		5% (1/22)			Pakistan	Farm	
			1996		5% (2/37)			Local	Farm	
			1998		3% (2/62)			Local	Farm: 0%	
			1999		27% (71/266)			Local	(0/50)	
			2000		32% (82/258)			Local	Nomadic: 17% (2/12)	
			2007		0% (0/28)			Local	Farm: 18% (32/175)	
			2008		56% (103/183)			Local	Nomadic: 43% (39/91)	
			2013		17% (8/47)			Local	Farm: 4% (4/112)	
									Nomadic: 53% (78/146)	
									Isolated	
									Nomadic	
									Farm: 3% (1/40)	
									Nomadic: 100% (7/7)	

References	Study design	Country of origin	Year	MERS-CoV RNA presence	MERS-CoV sero-prevalence	Sex	Age	Imported/local	Sampling location	Other animals tested
Haegmans et al. [3]	Human outbreak investigation	Qatar	2013	Nasal: 86% (12/14) Oral: 0% (0/14) Rectal: 0% (0/14)	IFA/V: 100% (14/14)			Farm		
Hemida et al. [19]	Longitudinal	KSA	2013-2014	Nasal: 33% (9/27) Oral: 0% (0/17) Rectal: 3% (1/37)			<2Y: 39% (7/18) 6-14Y: 22% (2/9)	Farm		
Hemida et al. [69]	Cross-sectional	KSA	1993		pp: 90% (118/131)			Farm		
Meyer et al. [37]	Cross-sectional	Australia	2014		pp: 0% (0/25)			Farm and abattoir (feral)		
		Egypt	2014		pp: 100% (7/7)			Abattoir		Bactrian camel 0% (0/16)
		UAE	2003		IFA: 100% (151/151)		>2Y: 100% (151/151)			
			2013		IFA: 96% (481/500)		2-8Y: 89% (89/100) >2Y: 89% (89/100)	Farm (racing): 89% (89/100) KSA, Sudan, Pakistan and Oman (217/218)		
Müller et al. [32]	Cross-sectional	Somalia	1983-1984		ELISA: 84% (72/86)			UAE	Isolated: 0% (0/5)	
		Sudan	1983		m: 81% (70/86) ELISA: 84% (159/189)	Female: ELISA 84% (159/189)	>6Y: 84% (159/189)	Farm		
		Egypt	1997		m: 81% (153/189) ELISA: 81% (35/43)					
Nowotny et al. [70]	Cross-sectional	Oman	2013		m: 79% (34/43)					
Raj et al. [71]	Cross-sectional	Qatar	2014		Nasal: 7% (5/76) Nasal: 2% (1/53)					

Table 1: Continued

References	Study design	Country of origin	Year	MERS-CoV RNA presence	MERS-CoV sero-prevalence	Sex	Age	Imported/ local	Sampling location	Other animals tested
Reusken et al. [28]	Cross-sectional	Qatar	2013	Nasal: 15% (5/33) Rectal: 9% (3/33) Milk: 15% (5/33)	pMA: 100% (33/33) Milk: pMA 100% (12/12), 75% (9/12)	Female: 15% (5/33)	>5y: PCR 42% (5/12) ELISA 100% (12/12)		Farm	
Reusken et al. [28]	Cross-sectional	Nigeria	2010-2011		pMA: 28% (100/358)		4-15y: 28% (100/358)	Abattoir also serves Chad, Niger, CAR	Abattoir	
		Tunisia	2009		pMA: 49% (99/204)		≤2Y: 30% (14/46) >2Y: 54% (85/158)			
		Ethiopia	2010-2011		pMA: 96% (181/188)		≤2Y: 94% (29/31) >2Y: 97% (152/157)		Farm	
Woo et al. [25]	Cross-sectional	UAE	2013	Fecal: 5% (14/293)	98% (58/59) IFA: 100% (59/59)		<1Y: PCR 21% (13/61) : 98% (54/55) ≥1Y: PCR: 0% (1/232) : 100% (4/4)		Farm	
Al Hammadi et al. [72]	Human outbreak investigation	UAE	2015	Nasal: 100% (8/8)	ppNT: 100% (5/5)	Female: 100% (5/5)	<1y: 100% (4/4) 10Y: 100% (1/1)	Oman	Border screening	
Chu et al. [73]	Cross-sectional	Nigeria	2015	Nasal: 11% (14/132)	ppNT: 95% (125/131) VNT: 0% (0/307)		>6y: 95% (125/131)		Abattoir	
Cramerli et al. [58]	Cross-sectional	Australia	2013-2014		pMA: 50% (166/335)		<6m: 36% (22/61) 6m-2Y: 30% (24/80) >2Y: 62% (120/194)		Abattoir: 231 Feral camel muster: 76 Farm: 48% (124/261) Nomadic: 57% (42/74)	
Deem et al. [40]	Cross-sectional	Kenya	2013							

References	Study design	Country of origin	Year	MERS-CoV RNA presence	MERS-CoV sero-prevalence	Sex	Age	Imported/local	Sampling location	Other animals tested
Farag et al. [26]	Cross-sectional	Qatar	2014	Nasal: 60% (61/101) Oral: 23% (23/102) Rectal: 15% (15/103) Bronchial: 7% (7/101) Lymph nodes: 9% (5/53)	pMA: 97% (100/103)		<1Y: PCR: 68% (50/73) ≥1Y: PCR: 39% (11/28)		Abattoir	
Gutierrez et al. [33]	Cross-sectional	Canary Islands	2015		ELISA: 4% (7/170)	Male: 0% (0/101) Female: 10% (7/69)	≥2Y: 4% (7/170) All positives were aged 20-26Y	African: 41% (7/17) Local: 0% (0/153)	Farm	
Khalafalla et al. [20]	Longitudinal	KSA	2013-2014	Nasal: 29% (28/96) Lung tissue 62% (56/91)			<4Y: 42% (15/36) ≥4Y: 22% (13/60)		Abattoir, live animal market, veterinary hospital	
Shirato et al. [47]	Cross-sectional	Japan	2015	Nasal: 0% (0/4) Rectal: 0% (0/18) Oral: 0% (0/10)	ELISA: 0% (0/5)	Male: Nasal PCR 0% (0/1) 0% (0/1) Female: Nasal PCR 0% (0/3) 0% (0/3)	<2Y: 0% (0/1) >5Y: PCR 0% (0/3) 0% (0/3)		Zoo	Bactrian camels: PCR: 0% (0/6) ELISA: 0% (0/6)
Wernery et al. [55]	Cross-sectional	UAE	2015	Nasal: 0% (0/254) Milk: 0% (0/1333)	ELISA: 92% (234/254)	Female: ELISA 99% (132/133)	0-3m: ELISA: 75% (24/32) 4m: ELISA: 79% (11/14) 5-6m: ELISA: 89% (41/46) 7-12m: ELISA: 90% (26/29) >12m: ELISA: 99% (132/133)		Farm	

Table 1: Continued

References	Study design	Country of origin	Year	MERS-CoV RNA presence	MERS-CoV sero-prevalence	Sex	Age	Imported/local	Sampling location	Other animals tested
Wernery et al. [55]	Cross-sectional	UAE	2015	Nasal: 5% (45/871)	ELISA: 93% (786/843)		<1Y: PCR: 35% (24/68) ELISA 85% (92/108) 2-4Y: PCR: 3% (10/344) ELISA 97% (328/340) >4Y: PCR: 0% (0/250) ELISA 96% (298/310)	KSA	Farm	
Yusof et al. [74]	Cross-sectional	UAE	2014	Nasal: 2% (126/7803)				KSA	Border (70/4617)	Border screening: 2% (70/4617)
								Oman	Border (31/2853)	Border screening: 1% (31/2853)
									(25/303)	Abattoir: 8% (25/303)
									Public escort and zoo: 0% (0/30)	Public escort and zoo: 0% (0/30)
Meyer et al. [30]	Longitudinal	UAE	2014-2015	At 6m (nasal): 18% (2/11) of calves, no dams	At day 0: MN/ ELISA 0% (0/11)		Dams: ELISA: 100% (11/11)		Farm	
	11 calf-dam pairs				Maternal Ab peak at day 7					
					At 5-6m: 45% (5/11)					
					At 12m: 100% (22/22)					
Miguel et al. [46]	Cross-sectional	Kazakhstan	2015		ppNT: 0% (0/455)	Female: 0% (0/455)			Farm	Bactrian camels: ppNT: 0% (0/95)
Muhairi et al. [29]	Human outbreak investigation	UAE	2014	Farms MERS patients (n=2): Nasal: 10% (15/155) Surrounding farms: Nasal: 3% (27/992)					Farm	Sheep: 0% (0/34)

References	Study design	Country of origin	Year	MERS-CoV RNA presence	MERS-CoV sero-prevalence	Sex	Age	Imported/ local	Sampling location	Other animals tested
Sabir et al. [22]	Cross-sectional	KSA	2014-2015	Nasal: 12% (159/1309) Rectal: 0% (0/304)			5-6m: 15% (28/190) 6m-1y: 18% (58/315) 1-2y: 8% (42/509) 2-4y: 10% (20/206) >4y: 11% (5/46)	Local: 15% (133/893) Sudan: 6% (14/133) Somalia: 7% (19/291)	Abattoir: 0% (0/14) Farm: 11% (7/116) Market: 12% (145/1162)	
			2015-2016	15% (15/100) (94 nasal, 6 oropharyngeal swabs)		Male: 18% (3/17) Female: 14% (12/83)	<1y: 0% (0/9) 1-5y: 15% (6/41) 5-10y: 16% (6/38) >10y: 25% (3/12)	Farm: 16% (13/80) Abattoir: 10% (2/20)		
Ali et al. [17]	Cross-sectional	Egypt	2014-2016	Nasal: 15% (435/2825) Rectal: 15% (18/114) Milk: 6% (12/187) Urine: 0% (0/26)	MN: 71% (1808/2541) Milk: 20% (38/187)	Male: PCR 21% (300/1439) MN: 72% (905/1254) Female: PCR 11% (115/1089) MN 66% (724/1090)	<2y: PCR 16% (97/591) 2y: PCR 10% (221/596) >2y: PCR 10% (228/2234) (1587/1945)	Local: PCR 12% (192/1658) MN 92% (159/172) MN 82% (1015/1655) Sudan, Somalia, and Ethio- pia: PCR 21% (243/1167) MN 90% (793/886)	Market: PCR 2.5% (4/159) MN 92% (159/172) Nomadic: PCR 1% (3/282) MN 72% (202/282) Farm: PCR 14% (189/1376) MN 59% (813/1373) Quarantine: PCR 36% (153/424) MN 95% (342/361) Abattoir: PCR 15% (86/584) MN 83% (292/353)	

Table 1: Continued

References	Study design	Country of origin	Year	MERS-CoV RNA presence	MERS-CoV sero-prevalence	Sex	Age	Imported/local	Sampling location	Other animals tested
Ali et al. [27]	Cross-sectional	Egypt	2014-2015	Nasal: 4% (41/1078)	MN: 84% (871/1031)	Male: PCR 3% (21/798) MN 85% (651/765) Female: PCR 7% (20/280) MN 83% (220/266)	<2Y: PCR 2% (2/82) MN 52% (42/81) >2Y: PCR 4% (39/996) MN 87% (829/950)	Local: PCR 1% (2/340) MN 76% (257/339) East Africa: PCR 3% (4/115) MN 72% (71/98) Sudan: PCR 6% (35/623) MN 91% (543/594)	Market: PCR 3% (9/290) MN 94% (273/289) Village: PCR 2% (1/51) East Africa: PCR 1% (2/340) MN 76% (256/339) Quarantine: PCR 2% (4/164) MN 96% (1557/164) Abattoir: PCR 9% (26/284) MN 77% (184/239) Farm PCR 77% (17/22) ELISA 78% (14/18) Nomadic: PCR (10/20) ELISA 87% (20/23)	Cattle: PCR 0% (0/35) MN 0% (0/35) Sheep: PCR 0% (0/51) MN 2% (1/51) Goat: PCR 0% (0/36) MN 0% (0/4) Buffalo: PCR 0% (0/4) MN 0% (0/15) Donkey: PCR 0% (0/15) Horse: PCR 0% (0/4) MN 0% (0/4) Bat: 0% (0/91) Cattle: ELISA 0% (0/5) Sheep: ELISA 0% (0/10)
Doremalen et al. [23]	Cross-sectional	Jordan	2016	Nasal: 67% (28/42) Rectal: 0% (0/42) Urogenital: 0% (0/42)	ELISA 82% (37/45)		<1Y: PCR 61% (11/18) 1-2Y: PCR 92% (12/13) 2-5Y: PCR 50% (5/10) ELISA 100% (10/10) >5Y: PCR 0% (0/1) ELISA 100% (4/4)	(184/239)	Farm PCR 77% (17/22) ELISA 78% (14/18) Nomadic: PCR (10/20) ELISA 87% (20/23)	Cattle: ELISA 0% (0/5) Sheep: ELISA 0% (0/10)
Faizarano et al. [24]	Cross-sectional	Mali	2009-2010		ELISA: 88% (502/571)	Male: 86% (210/245) Female: 92% (302/328)	1-2Y: 83% 3-8Y: 91% 9-16Y: 88%		Farm	Cattle and sheep: 0% (0/10)
Hemida et al.	Longitudinal	KSA	2014-2015	Nasal: 4% (3/70) Rectal: 0% (0/70)	ppNT: 100% (70/70)		<2Y: 19% (3/16) >2Y: 0% (0/39)		Farm	

References	Study design	Country of origin	Year	MERS-CoV RNA presence	MERS-CoV sero-prevalence	Sex	Age	Imported/ local	Sampling location	Other animals tested
Kasem et al, 2017 [38]	Human outbreak investigation	KSA	2014-2016	Nasal: 10% (75/780) (camels with MERS patients contact)	ELISA: 71% (422/595)	Male: PCR 20% (49/245) ELISA 84% (127/152) Female: PCR 5% (26/535) ELISA 67% (295/443)	≤2Y: PCR 15% (46/298) 1-4Y: PCR 6% (13/202) 4-6Y: PCR 4% (6/144) >6Y: PCR 7% (10/136)	Farm		
Miguel et al. [39]	Cross-sectional	Burkina Faso Ethiopia Morocco	2015	Nasal: 5% (27/525) Nasal: 11% (70/632) Nasal: 1% (5/343)	ppNT: 80% (421/525) 95% (600/632) 77% (265/343)	Seropositivity and CR positive rate higher in females	Seropositivity rates increased, MERS RNA detection rate decreased with age			
Munyua et al. [76]	Cross-sectional	Kenya	2013		ELISA 90% (789/877)	Male: 81% (173/213) Female 93% (616/664)	1-4Y: 73% (209/285) 4-6Y: 99% (116/117) >6Y: 98% (466/476)	Farm: 71% (10/14) Nomadic: 91% (698/771)		
Saqib et al. [35]	Cross-sectional	Pakistan	2012-2015		ELISA: 56% (315/565) MN: 39% (223/565)	Male: ELISA/ MN: 44% (96/217) Female: ELISA/ MN: 36% (127/348)	≤2Y: MN 29% (26/89) 2-5Y: 30% (62/208) 5-10Y: 51% (92/180) >10Y: 49% (43/88)			
Yusof et al. [41]	Cross-sectional	UAE	2015	Nasal: 29% (109/376)		Male: 27% (73/269) Female: 31% (33/107)	<1Y: 32% (81/255) >1Y: 21% (25/121)	Local: 25% (53/210) Market Oman: 50% (53/106) 5% (3/60)		
Li et al. [76]										

Table 1: Continued

References	Study design	Country of origin	Year	MERS-CoV RNA presence	MERS-CoV sero-prevalence	Sex	Age	Imported/ local	Sampling location	Other animals tested
David et al. [43]		Israel	2012-2017 (serum)	Nasal: 0% (0/540)	VNT: 62% (254/411)	Male: PCR 0% (0/54) Female: PCR: 0% (0/486)			Farm	Llama PCR 0% (0/19) ELISA: 37% (7/19) VNT: 32% (6/19) Alpaca PCR 0% (0/102) ELISA 34% (35/102) VNT: 32% (30/102)
Chu et al. [65]	Cross-sectional	Ethiopia	2016-2017 (nasal swab)	Nasal: 5% (5/102)						
Harrath et al. [78]	Cross-sectional	KSA	2016		ELISA: 84% (144/171)	Male: 83% (77/93) Female: 87% (68/78)	<2Y: 93% (66/71) 2-5Y: 78% (78/100)	Local	Farm	
Islam et al. [34]	Cross-sectional	Bangladesh	2015	Nasal: 0% (0/55)	ELISA/ppNT: 31% (17/55)	Male: ppNT 34% (10/29) Female: ppNT 27% (7/26)	<2Y: ELISA/ppNT 9% (1/11) ≥2: ELISA/ppNT 36% (16/44)	Local: ELISA/ppNT 4% (1/24) India: ELISA/ppNT 52% (16/31)	Market: 63% (12/19) Farm: 14% (5/36)	Sheep: PCR 0% (0/18) ELISA/pp 0% (0/18)
Kasem et al. [79]	Cross-sectional	KSA	2015-2017	Nasal: 56% (394/698)			<2Y: 72% (303/423) >2Y: 33% (91/275)	Market: 42% (184/435) Abattoir: 80% (210/263)		

Age

The seroprevalence of MERS-CoV antibodies increases with age in camels, while the fraction of camels that test positive for MERS-CoV RNA in their nasal swabs decreases with age [17, 31, 36, 38, 39]. When all serological results of papers that included sufficient age information is combined, the median seroprevalence of camels aged under 2 years is 52% (992/1972; range 0–100%), while the age groups 2–5 years (702/924; range 30–100%) and over 5 years old (1226/1370; range 0–100%) had a combined median seroprevalence of 97%. In the virological studies reporting age breakdown, the median rate of nasal shedding in 0–2 years old camels was 34% (718/2612; range 0–100%) of cases, compared with 2% (91/1142; range 0–100%) in camels older than 2 years.

Sex

Some individual studies show a significantly higher seroprevalence in female camels compared with males [27, 39], while others show the opposite [38] or do not find any significant difference [17, 35]. Similar disagreeing results are published for the presence of MERS-CoV RNA in male vs. female camels [17, 27, 38, 39]. In the studies in this review where sex of camels was recorded, a total of 4810 serum samples from female camels and 3458 samples from male camels were collected and analysed for MERS-CoV antibodies, compared with 2007 vs. 2505 nasal swabs for viral RNA testing. Approximately three times more female camels were sampled at farms, while male camels were in the majority in studies that looked at MERS-CoV prevalence of camels at slaughterhouses, live animal markets and quarantine areas. The overall median Seroprevalence of male and female camels in our review is 50% and 67%, respectively (range 0–100%; excluding results from Israel and Kazakhstan). The median percentage of presence of viral RNA is 18% in nasal swabs of male camels (range 0–21%) compared with 9% in female camels (range 0–100%), in our review.

Sampling location and herd characteristics

In several studies, camels that were sampled at animal markets or quarantine facilities were seropositive more often than camels at farms [17, 22, 27, 34]. Combining serological laboratory results of camels in our review with sufficient background information with regard to the sampling location does not result in the same pattern, with a median seroprevalence of 84% (5632/8115; range 0–100%; excluding Australia and Spain) in camels from farms and 80% (943/1005; range 28–98%) in the camel population sampled at markets and quarantine facilities. Studies in Egypt found a significantly higher PCR positivity rate in camels sampled in abattoirs or quarantine facilities, but these results could not be confirmed by other papers in this review [17, 27]. When comparing differences in seroprevalence or virus RNA positive rate in nomadic vs. sedentary camel herds, some authors did not find a statistical difference between the two herd management types [39, 40], while others found some evidence of higher seroprevalences in nomadic herds [27, 36]. One study in Kenya looked at

Table 2: Camel population (>10.000) and trade

Country	Camel population (OIE, 2016)	Camel density (OIE, 2016) (Animals per square kilometer)
Africa		
Algeria	354.565 (OIE, 2014)	0.15 (OIE, 2014)
Burkino Faso	19.097	0.07
Djibouti	50.000	2.17
Egypt	66.233	0.07
Eritrea	385.283	3.18
Ethiopia	1.200.000	1.06
Kenya	2.986.057	5.12
Libya	110.000	0.06
Mali	1.028.700	0.83
Mauritania	1.379.417 (OIE, 2013)	1.34 (OIE, 2013)
Morocco	197.550 (OIE, 2014)	0.44 (OIE, 2014)
Niger	1.698.110 (OIE, 2013)	1.34 (OIE, 2013)
Nigeria	279.397	0.3
Sudan	4.830.000	1.93
Somalia	7.100.000	11.13
Chad	6.400.000	4.98
Tunisia	56021	0.34
Middle East/Central Asia		
Afghanistan	175.270	0.21
Indiab	400.000 (OIE, 2015)	0.12 (OIE, 2015)
Iranb	171.500	0.10
Iraq	81.205	0.19
Jordan	10.872 (OIE, 2014)	0.12 (OIE, 2014)
Kazakhstan b	170.513	0.06
Kuwait	80.790	4.53
Oman	257.713	1.21
Pakistanb	1.000.000	1.24
Qatar	77.417 (OIE, 2014)	6.77 (OIE, 2014)
Saudi Arabia	481.138	0.25
Syria	45.610	0.25
Turkmenistanb	122.900	0.25
UAE	392.667	4.74
Uzbekistanb	14.800	0.03
Yemen	459.366	0.87

Excluding China and Mongolia because the large majority of camel population are Bactrian camels

Camel population exists of both dromedary and Bactrian camels (L. Ming, L. Yi, 1 R. Sa, 1 Z. X. Wang, Z., Genetic diversity and phylogeographic structure of Bactrian camels shown by mitochondrial sequence variations, Anim Genet. 2017 Apr; 48(2): 217–220)

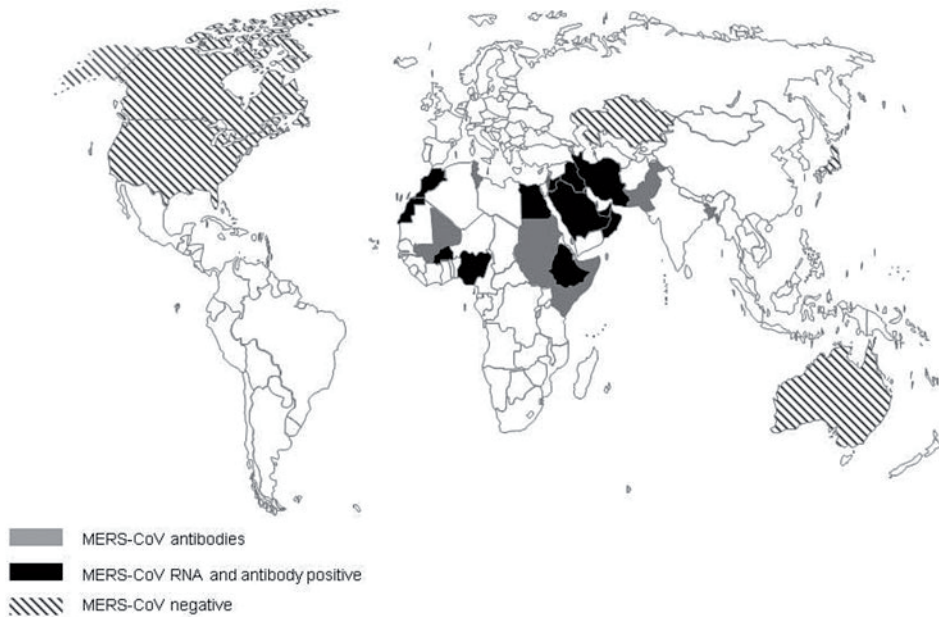


Figure 2: Virological and serological evidence for MERS CoV in dromedary camels.

the differences between herds with different levels of isolation, and did not find significant differences in MERS-CoV antibody levels [40].

Animal origin

Most studies that compared local camels with imported camels suggested that imported camels are seropositive for MERS-CoV more often [9, 17, 27, 34, 41], although not all differences were significant. Two studies in Egypt found a significantly higher RNA positivity rate in imported camels from East Africa compared with domestically bred camels [17, 27], while another study executed in the KSA found a significantly higher number of MERS-CoV RNA-positive results amongst local camels vs. camels from Sudan and Somalia [22].

Seasonal variation in MERS-CoV circulation in the camel population

Although MERS-CoV was detected almost year-round in camels, some studies show a relatively higher seroprevalence and viral detection during the cooler winter months [17, 20, 27, 38].

MERS-CoV in non-dromedary animals

MERS-CoV antibodies have been detected in llamas and alpacas in Israel and in alpacas in Qatar [42, 43]. To date, no MERS-CoV antibodies or viral RNA have been detected in Bactrian camels [4, 37, 44–47] (Table 1 and Table 3). Swine, goats and horses that were included in the field surveys in our review all tested negative for MERS-CoV RNA and antibodies [4, 17,

Table 3: MERS-CoV in non-dromedary animals in the field

Species	Seroprevalence	Viral RNA ^c
Bactrian camel	0% (0/505) (Oman, UAE, Mongolia, China, Kazakhstan)	0/390 (China, Mongolia)
Alpaca	24% (30/126) (Israel(+), Oman) 100% (15/15), Qatar ^b	0% (0/102) (Israel) 0% (0/15)(Qatar) ^b
Llama	23% (6/26) (Israel (+), Oman)	0% (0/19) (Israel)
Guanaco	0% (0/2)	-
Cattle and Buffalos	0% (0/258) (KSA, Egypt, Oman, Jordan)	0% (0/35) (Egypt)
Swine	0% (0/260) (Egypt)	-
Sheep	0.2% (1/482) ^a (KSA, Egypt (+), Oman, Jordan, UAE, Bangladesh)	0% (0/307) (Jordan, KSA, UAE, Egypt, Bangladesh)
Goats	0% (0/399) (KSA, Egypt, Oman, Jordan)	0% (0/72) (KSA, Egypt)
Horses, donkeys	0% (0/22) (Egypt, UAE) 0% (0/192)(UAE) ^b	0% (0/19) (Egypt)
Birds	0% (0/444) (KSA, Hong Kong)	-
Bats	0% (0/91) (Egypt)	-

a. 6 additional sera from sheep in Qatar tested positive by protein microarray (pMA), but could not be confirmed by NT

b. Articles that were not included in the original literature search, because no dromedary camels were investigated in these studies

c. MERS-CoV RNA in nasal swabs

31, 48–52]. MERS-CoV antibodies were detected in two studies in sheep in Egypt and Qatar, although in very low numbers [17, 51]. However, most surveys that investigated sheep did not find evidence of MERS-CoV infection or exposure [4, 23, 29, 31, 34, 48–51, 53].

DISCUSSION

The publications in this review show that the MERS-CoV mainly circulates in dromedary camel populations in the Middle East and part of Africa, and has been infecting dromedary camels in Africa for more than three decades. Antibodies have also been found in Arabic camel sera from the early 90s [31, 32]. However, MERS-CoV was discovered until 2012, after the first human cases appeared [1], which is probably due to the minor clinical symptoms of MERS-CoV infections in camels [18]. Most camel surveys were conducted in the Middle

East and some northern and eastern African countries, but significant data gaps currently still exist in the north and west of Africa, in countries that have camel populations of 100 000 to more than a million animals, such as Algeria, Libya, Mauritania and Niger. Even less is known about the central Asian region. Some evidence of MERS-CoV circulation in camels of Pakistan and Bangladesh was recently published, but data is lacking from Afghanistan and India. Knowledge on the presence of MERS-CoV in the animal reservoir is a crucial first step to assess whether MERS-CoV could be a relevant public health threat in these regions.

MERS-CoV infections are mainly detected in calves and young camels [30, 31]. The research included in this review shows that the IgG positivity rate increases gradually in dromedary camels of increasing age while the MERS-CoV RNA detection rate decreases. Maternal IgG antibodies in camels are acquired through the intake of colostrum during the first 24 h post-parturition. After 24 h, antibody levels in the dam's milk decrease rapidly [54]. One study showed that maternal antibodies in calves peak at 7 days post parturition and decline in the following 6 months. After 5–6 months, over half of the calves did not have maternal neutralizing antibodies in their serum any longer [30]. However, in other field studies, the titre of MERS-CoV-specific antibodies is still low at 1 month of age and increases with age in dromedary calves [27, 55]. A lower or undetectable antibody levels in young camels is likely to explain the higher MERS-CoV RNA detection rate. In adult camels, a much higher MERS-CoV seroprevalence can be found, which is probably due to a long-lasting immune response against a MERS-CoV infection or multiple re-infections with MERS-CoV. Immunity is not sterilizing, as MERS-CoV infection and shedding have also been shown in adult camels that have MERS-CoV antibodies [19, 21, 23, 24, 30, 31]. Several articles have analysed seroprevalence and virus shedding data in relation to factors, other than age, that may explain differences in seroprevalence and MERS-CoV RNA-positive rate in camels, such as sex, sampling location, herd characteristics and animal origin. Our review shows that there is considerable heterogeneity in results. In addition, comparison between studies is difficult given the lack of standardisation of study designs. A key factor to consider when comparing studies is the difference in distribution of male and female camels amongst different disciplines of camel husbandry. Females are mainly used for milking and reproduction. As a result, they often stay at farms. Male camels, especially of young age (<1 year old), are the predominant sex in slaughterhouses and amongst camels used for transport [39, 56]. This also influences the risk profile of acquiring a MERS-CoV infection. Female camels are in closer contact with calves, who are more susceptible to infection and shed virus in higher quantities compared with older camels [30]. On the other hand, meat and transport camels (predominantly male) travel more, leading to increased contact with other camels and camel herds, and therefore a higher chance of exposure to MERS-CoV. Some papers in this review suggest that there is a generally lower infection rate of domestically bred camels and camels on farms compared with imported camels and camels on animal markets or in quarantine facilities. This may be explained by the same increased contact rate and mixing of camel herds, leading to an increased chance

of MERS-CoV exposure and spread. The increase in MERS-CoV circulation in winter and spring can have multiple explanations. Firstly, the winter is the calving season [10], which leads to a larger proportion of young animals that usually have a higher number of MERS-CoV infections and virus excretion. Moreover, in winter season, there is a major increase of camel and human movements due to camel racing competitions, camel breeding, trading and movements to grazing grounds, which increases the chance of virus spread. Additionally, cooler temperatures may facilitate coronavirus survival in the environment [57]. In experimental studies, llama's and alpaca's are shown to be susceptible to infection with MERS-CoV [58, 59], which was confirmed by two papers in our review, describing serologically positive llamas and alpacas in Israel and alpacas with MERS-CoV neutralizing antibodies in Qatar [42, 43]. In experimental settings, animal-to-animal transmission has been shown for alpacas, making them a possible risk population for human infections [58]. Two studies in our review also found anti-MERS-CoV antibodies in sheep [17, 51] but experimental inoculation of sheep did not result in MERS-CoV replication or antibody development [59, 60]. However, the DPP4 receptor, the entry receptor for MERS-CoV, is present in sheep tissues, making it possible for the virus to bind to the sheep respiratory tract which may explain the finding of MERS-CoV antibodies [61]. Pigs also express the DPP4 receptor in their respiratory tract, and viral replication in experimental settings has been shown for pigs, but no antibodies or MERS-CoV RNA have been found in pigs during field surveys [48, 59]. This may be explained by the limited viral shedding in pigs and the absence of animal-to-animal transmission [62, 63]. We show that dromedary camels are present in large parts of the African and Asian continent, and that MERS infections in dromedary camels are widespread. However, human infections due to spill-over from the dromedary camel reservoir have not been reported in Africa [10]. Several explanations for the difference in human cases between the Arabian Peninsula and Africa have been suggested, such as differences in cultural habits, camel husbandry, prevalence of comorbidities, under detection or genetic factors in the local population [64]. Moreover, West African viruses were found to be phylogenetically and phenotypically distinct from the MERS-CoV viruses that caused human disease in the Middle East [65]. Increased knowledge on the animal reservoir of MERS-CoV needs to be combined with research on MERS prevalence and risk factors in humans to assess the true public health risk. Moreover, the absence of human disease, combined with the mild symptoms in camels, caused by MERS, will likely have a negative effect on the willingness to implement interventions and the cost-effectiveness of possible interventions in some areas.

CONCLUSION

Since the discovery of MERS-CoV in 2012, the dromedary camel has been identified as the animal reservoir of human infections with the MERS-CoV. However, the exact route

of human primary infections is still unknown. Moreover, the scale of the spread and prevalence of MERS-CoV in the camel reservoir is not fully known yet since there is still a lack of MERS-CoV prevalence data in some countries that harbour a very significant proportion of the world camel population. However, knowledge of the animal reservoir of MERS-CoV is essential to develop intervention and control measures to prevent human infections. Prospective studies that include representative sampling of camels of different age groups and sex, within the different husbandry practices, are needed to fully understand the patterns of MERS-CoV circulation. Such studies are important as they may give more information on critical control points for interventions to reduce the circulation of MERS-CoV and/or exposure of humans.

Author ORCIDs. R. S. Sikkema, 0000-0001-7331-6274

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Conflict of interest

None.

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Chapter 9

Drivers of MERS-CoV emergence in Qatar

FARAG, E., SIKKEMA, R. S., VINKS, T., ISLAM, M. M., NOUR, M., AL-ROMAIHI, H., AL THANI, M., ATTA, M., ALHAJRI, F. H., AL-MARRI, S., ALHAJRI, M., REUSKEN, C. & KOOPMANS, M. 2018. Drivers of MERS-CoV Emergence in Qatar. *Viruses*, 11.

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ABSTRACT

MERS-CoV (Middle East respiratory syndrome corona virus) antibodies were detected in camels since 1983, but the first human case was only detected in 2012. This study sought to identify and quantify possible drivers for the MERS-CoV emergence and spillover to humans. A list of potential human, animal and environmental drivers for disease emergence were identified from literature. Trends in possible drivers were analyzed from national and international databases, and through structured interviews with experts in Qatar. The discovery and exploitation of oil and gas led to a 5-fold increase in Qatar GDP coupled with a 7-fold population growth in the past 30 years. The lifestyle gradually transformed from Bedouin life to urban sedentary life, along with a sharp increase in obesity and other comorbidities. Owing to substantial governmental support, camel husbandry and competitions flourished, exacerbating the already rapidly occurring desertification that forced banning of free grazing in 2005. Consequently, camels were housed in compact barns alongside their workers. The transition in husbandry leading to high density camel farming along with increased exposure to humans, combined with the increase of camel movement for the racing and breeding industry, have led to a convergence of factors driving spillover of MERS-CoV from camels to humans.

Keywords: Drivers, MERS-CoV, Qatar

INTRODUCTION

Emerging infectious diseases are a cause for increasing global concern, because of their impact on global health and economics [1]. The Ebola outbreak in West Africa during 2014-2015 showed that pathogens which previously caused small and easy to control outbreaks had the potential to infect thousands of people under the right circumstances [2]. This is also a concern for the Middle East Respiratory Syndrome coronavirus (MERS-CoV), which until now has been the cause of sporadic cases and hospital outbreaks [3]. To date, there have been 2220 confirmed laboratory cases worldwide, with 790 deaths [4]. All MERS index cases are linked to the Arabian Peninsula. Dromedary camels have been identified as a reservoir of MERS-CoV with occasional zoonotic transmission to humans [5,6]. Human-to-human transmission is also common, with around 30% of the MERS cases reported to WHO being health care associated [7,8]. However, the source of infection of many index cases remains unclear [9,10].

Studies have shown that MERS-CoV, or related viruses have been circulating among camels at least since 1983 [11]. Since that period, massive changes have occurred in people's lives and in animal husbandry across the Arabian Peninsula. Understanding these changes may help to reconstruct the events that led to the emergence of MERS-CoV as a human disease. Past research identified several drivers of emerging zoonoses, such as urbanisation, population growth and demography, and environmental and agricultural changes [12,13,14]. The drivers which could have potentially influenced the MERS-CoV emergence in humans have only sporadically been investigated [15,16]. By reviewing changes involving humans and camels over the past 30 years in Qatar, this study sought to identify the key drivers of the emergence and spread of MERS-CoV.

METHODS

Potential drivers for disease emergence were identified from literature and from discussions with national and international experts in MERS-CoV. The final list had the following categories: economic development; human demography and behavior; international travel, commerce, sports and leisure; political environment; agriculture and food industry change, including camel demography, husbandry and movement; changes in climate and land use. Data from 1980 onwards were collected from national and international databases. If multiple data sources were available, data from both sources were collected. All data were entered in an excel datasheet and reviewed and discussed with the project team (Supplementary 1).

Qualitative information and remaining data gaps were addressed by interviews with a group of 15 experts and stakeholders from Qatar. Criteria to select experts included 5 years or more experience in a camel-related business (farming, trading and racing) or

professional services related to camels and being familiar with cultural aspects of the Qatari community. Using a structured interview guide (Supplementary 2) and a moderator, a series of 4 interviews were conducted in Arabic, each lasting approximately for 3 hours. The main themes that were covered during the interviews included: (changes in) people's living conditions; customs and purposes of camel ownership; cultural habits related to camels; educational level and personal behaviors of camel owners and workers; camel movement; demographic distribution of camels in Qatar; camel farming practices: feeding, grazing, and slaughter. A detailed transcript was shared with the experts for authentication. A literature search was done to complement findings from the quantitative and qualitative study, using PubMed, Google Scholar and the local sources of information including the Ministry of Public Health (MoPH), Ministry of Municipality and Environment (MME), Ministry of Development and Planning Statistics (MDPS), and Qatar Statistical Authority (QSA).

The funder had no role in study design, data analysis, data interpretation, or writing of the review.

RESULTS

Changes in the Economic Situation

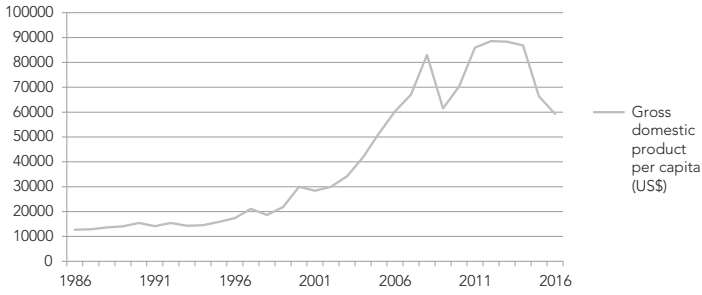
Historically, Qatari inhabitants were mostly Bedouins along with a few settled people [17,18]. The Bedouins owned limited numbers of camels, sheep, and goats [19]. Camels were used as a source of food (milk and meat) and means for transportation. In 1939, oil and natural gas resources were discovered. However, large-scale exploitation started in the 1950s [20]. From the 1950s onwards, Qatar's economy has been steadily growing. However, the year 2000 marked a significant turning point as Qatar's GDP almost increased by more than 5-fold during the period 2000–2006 (Figure 1A) [20,21]. Qatar is currently considered to be one of the wealthiest countries in the world [20].

Changes in Human Demography and Health

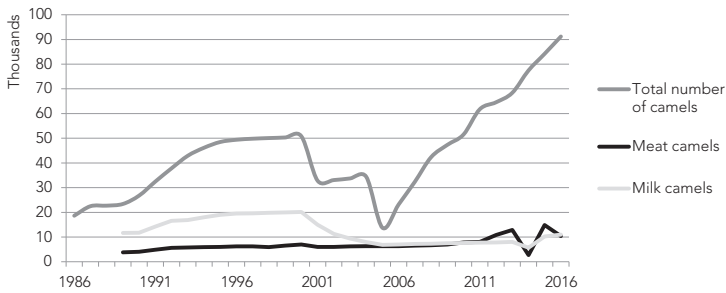
The thriving economy was paralleled by major demographic and life style changes. In the late 1950s, around 16,000 people lived in Qatar [22]. In response to demands for a larger workforce after the exploitation of oil and gas began, foreign laborers started to migrate to Qatar from countries in the region, like Palestine, Oman, Iran, and the Kingdom of Saudi Arabia (KSA). Later, immigrants from Pakistan, India, Nepal, Sri Lanka, Bangladesh, the

Figure 1: Developments in economy, camel demography, production, and trade. (A). Development over time of the gross domestic product per capita; (B) Development over time of the camel population; (C) Development over time of camel import and export; (D) Development over time of camel importation per country of origin. *Other Arab countries: Algeria, Comoros, Djibouti, Egypt, Iraq, Jordan, Lebanon, Libya, Mauritania, Morocco, Palestine, Somalia, Sudan, Syria, Tunisia, and Yemen. **Other GCC countries: Bahrain, Kuwait, Oman, Qatar.

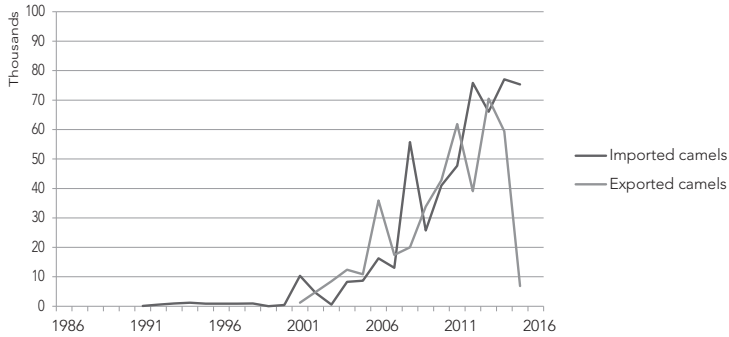
2A. Gross domestic product per capita



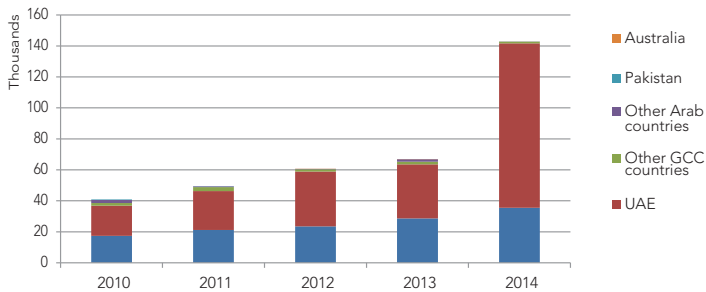
2B. Camel population



2C. Camel import & export



2D. Camel import : origins



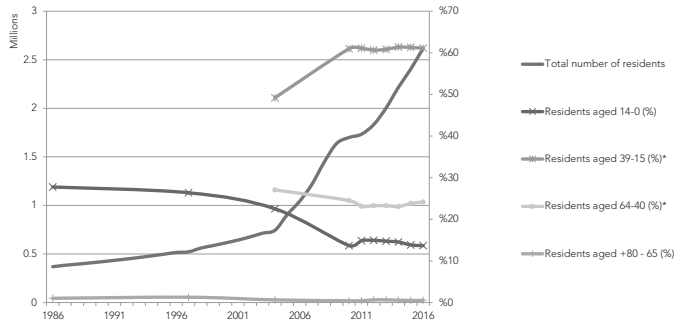
Philippines, and Indonesia joined the older migrant populations, increasing the number of inhabitants to 369,079 by 1986 and recently to 2,617,634 (Figure 2A) [23]. In 2016, non-Qatari males made up 78% of the residents of working age (15-64) and non-Qatari made up more than 90% of the total number of Qatar inhabitants older than 15 years of age (Figure 2B,C) [24]. Most recent estimations of the origins of the non-Qatari population are that 25% is Indian, 11% Bangladeshi, 14% Nepali, 10% Filipinos, 9% Egyptian, 5% Pakistani, and 2% Iranian [25]. The total number of males in Qatar increased from 67.2% of the total population in 1986 to 75.5% in 2016 (Figure 2B). In 2004, almost 50% of residents were between 15 and 39 years old, and this has risen to more than 60% in 2015 (Figure 2A). Detailed accounts on age distribution were not available before 2004 [26].

Most people in Qatar live in urban areas. The percentage of residents living in cities increased from 85.3% in 1960, to 90.4% in 1986, and 99.3% in 2016 [20]. Doha, the capital and the biggest city of Qatar, hosts the greatest number of people. However, there has also been a large increase in number of people living in the Al-Rayan area, where most of the camel farms are located. The number of tourists visiting Qatar also increased, especially since 2000. Most tourists came from other GCC countries, but the number of visitors from Europe and America were also increasing (Figure 2D) [20,27].

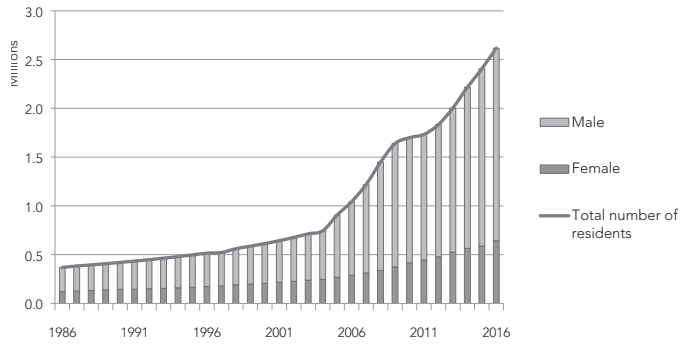
According to experts, the economic development and population increase coincided with major changes in life style. The Bedouin nomadic lifestyle gradually decreased as most of the Qatari tribes shifted to an urban, settled lifestyle; cars and planes rapidly replaced camels as transportation means. This transformation to a more sedentary lifestyle is reflected in the profile of comorbidities. More than 70% of adults are overweight and almost half of them obese [28]. Male obesity increased from 17% in 1986 to 34% in 2014, which is extremely high compared to the current 11% prevalence in men worldwide [29]. In 1998, 7% of residents above 15 years were hypertensive, rising to 14% in 2006, and 33% in 2012 [30,31]. Prevalence of high blood sugar among adults in 2015 was 14%, compared to a worldwide prevalence of 9% [28]. The Qatar Stepwise Report reported in 2012 that 15% of adults were daily smokers. Yet, Qatar has a low death rate: 1.49/1000, compared to the worldwide death rate of 7.72/1000, and its healthcare system has developed rapidly over the past twenty years [28,31,32].

Figure 2: Developments in human demography in Qatar; (A) Development over time of the age structure of the population; (B) Development over time of the human sex ratio; (C) Development over time of the ration Qatari vs. non Qatari; (D) Development over time of tourism per country of origin *Other Arab countries: Algeria, Comoros, Djibouti, Egypt, Iraq, Jordan, Lebanon, Libya, Mauritania, Morocco, Palestine, Somalia, Sudan, Syria, Tunisia, and Yemen. **GCC countries: Bahrain, Kuwait, Oman, Qatar, KSA, and the United Arab Emirates

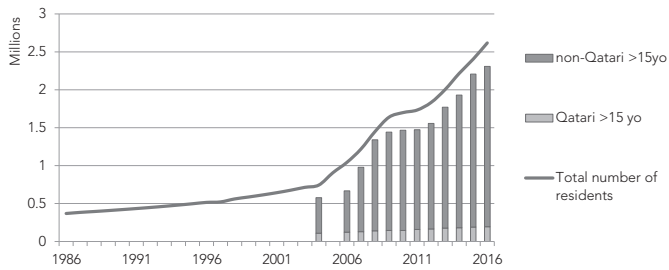
1A. Age



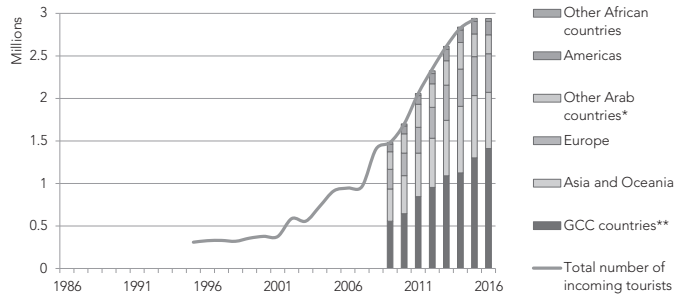
1B. Gender



1C. Nationality



1D. Tourism



Changes in Camel Husbandry and Practices

The increase in the number of dromedary camels reflects the increasing popularity of camels as sports animals (Figure 1B). With the changing life style and increasing wealth, the purchase and breeding of (expensive) racing camels came within reach of an increasingly large segment of the Qatari national population. According to experts, although camel racing has traditionally been part of the Bedouin culture, the organized racing business went through major changes over the past decades. This was partly due to financial and regulatory support from the Qatari government. This support increased the social and economic value of camels in Qatar, further stimulating their popularity. The Al Sheehaniya camel-racing track, one of the biggest tracks in the Gulf, was opened in 1990 [33]. The camel farms that are located near the Al Sheehaniya camel racing area are mostly used for racing camels. There are about 1500 racing camel holdings at the Al Sheehaniya camel racing area. Some of the camels in Qatar are used to compete in camel beauty contests that are organized around the Arabian Peninsula. According to the FAO, in 1960 there were about 6,000 camels in Qatar. This rose to over 43,000 in 1992, 50,000 in 2000, and more than 90,000 in 2016 (Figure 1B). More than 83% of the animals are currently kept for racing [34]. Across the Gulf region, Qatar has the highest camel density, with 6.77 units/km², compared to 4.74 units/km² in United Arab Emirates (UAE) and 0.11 units/km² in the KSA [35]. In 2005, the total number of camel farms was 1300 and by 2014 it had increased to 9594 [34].

As a result of the loss of traditional methods of rangeland management, the vegetation coverage decreased from 10% to only 1% of total land cover. Overgrazing of the green areas due to the increased population of camels and other livestock accelerated the desertification of Qatar [36]. Therefore, the government decided to assign natural protected areas in 2004 [37,38], and started to sanction the free grazing of livestock since 2005 [39]. By 2011, open grazing was completely banned [40]. According to the experts' opinions, this led to changes in farming practices, as herds were then moved outside of Qatar to areas where free grazing remained possible. Moreover, in Qatar, camels are now raised in closed systems and within 1 of 9 designated farming areas (camel complexes) in the residential districts. Camel workers also live on the premises of the camel complexes. Typically, a camel complex has a reception room (Majlis) for social activities of the camel owners. The Al-Rayyan municipality, where the Al Sheehaniya camel racing area is also located, currently holds about 83% of the total camel population and 61% of camel holdings (Figure 3) [34,35]. According to the experts, this newly adopted closed farming system led to the increase of disease incidence, especially of parasitic diseases. However, we did not find any disease statistics to substantiate these findings.

Changes in Race Camel Farming and Practices

The increasing focus on camel race competitions caused big changes in camel farming practices. Previously, the calves were weaned when the next calf was born. Currently,

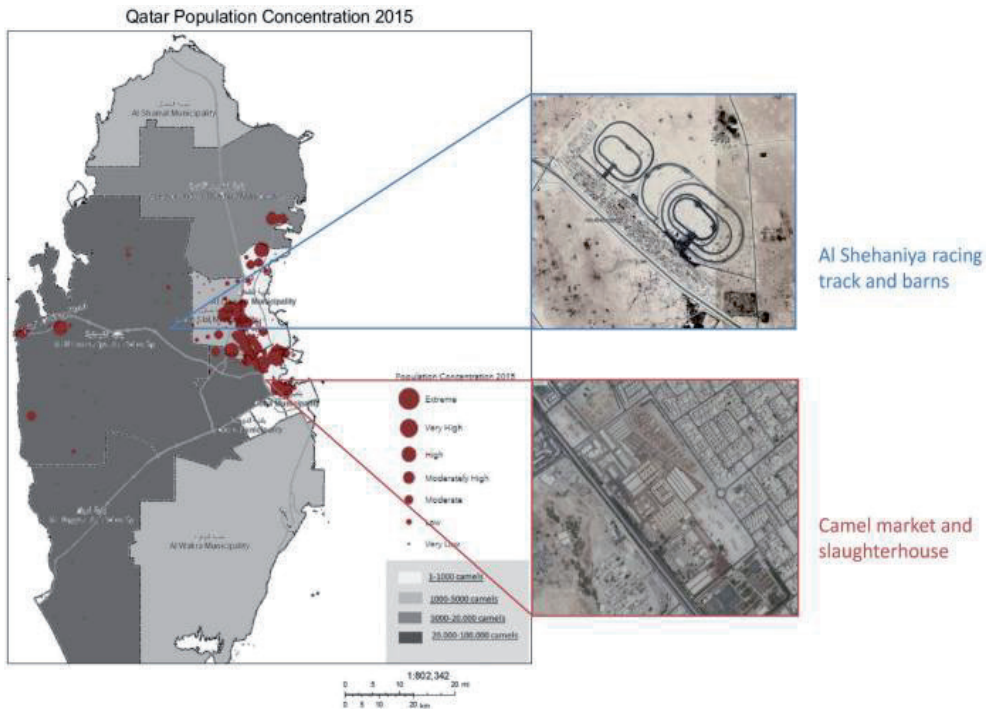


Figure 3: Human and camel density map of Qatar (adapted from Ministry of Development Planning and Statistics, Population Concentration map, 2015). The density map shows the density of camels (source: Ministry of Municipality and Environment) and humans in Qatar. Most people live in and around Doha, where the Doha animal market and slaughterhouse are also located. The highest camel density can be found in the Al-Rayyan area, where the Al Sheehaniya racing tracks are also located. A small, but growing, part of the Qatar population also lives in the Al-Rayyan area.

weaning occurs at around 7 months of age. After being weaned, young camels are directly taken for acclimatization (during the period mid-July through mid-August) from the general livestock farms (located across the region) to the racing farms, mostly located within the Al Sheehaniya area. This involves drastic changes in feeding systems, intense training for races, and mock races alongside camels from other farms and older training camels. The off-season for camel racing is during summer (mid-April to August) (Figure 4). During this time, most of the owners travel abroad, the frequency of visits to the farms substantially decreases, and workers are permitted to take annual vacations. From September onward, training intensifies, in preparation of the racing season, which lasts from mid-September through mid-April. During that time, 14,000 registered camels from different origins, ages, gender, nationalities, and breeds compete together at the Al Sheehaniya camel-racing track. During the racing season, up to 24 rounds take place, approximately five days per week.

Changes in International Camel Movements and Travel

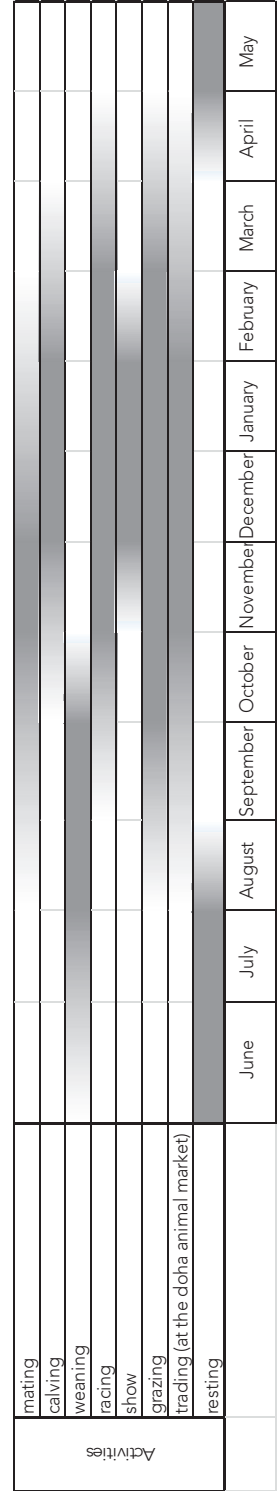
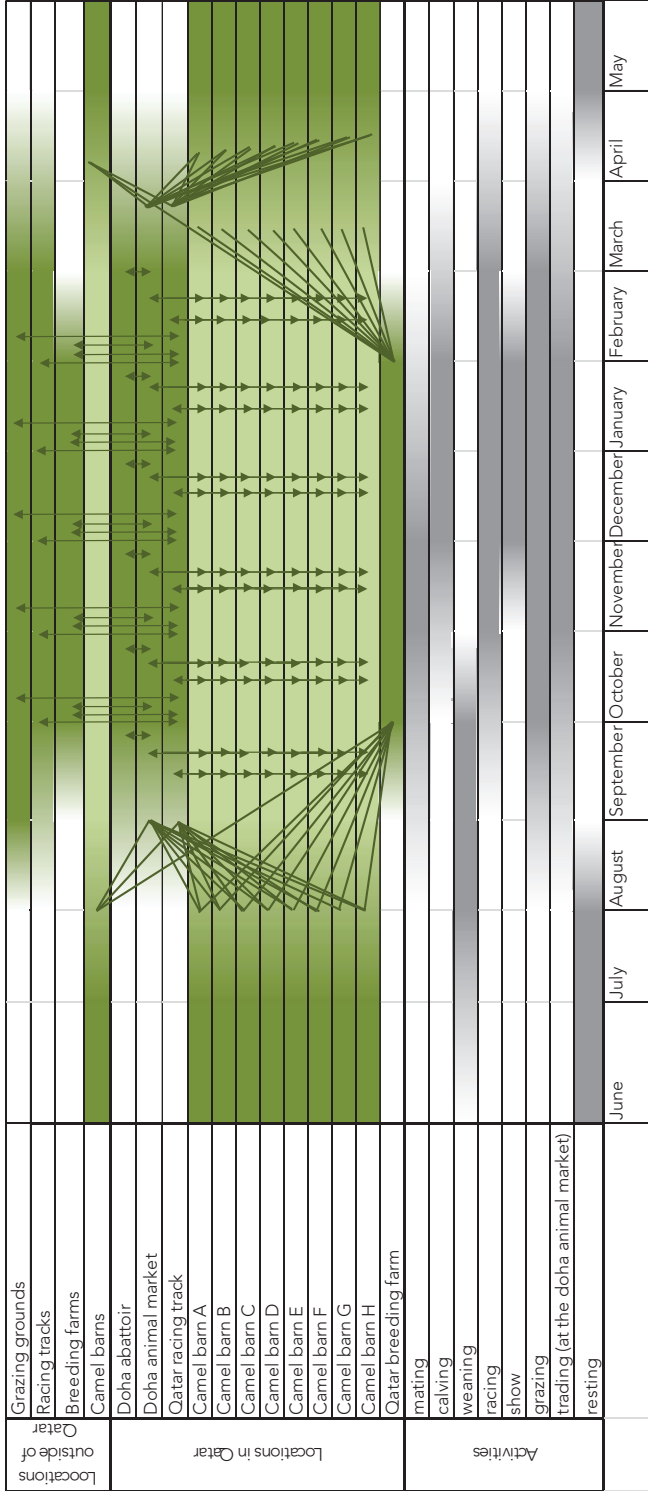
An unprecedented, increasingly intensified mobility of camels inside and outside Qatar has been seen over the recent decades. The domestic and cross border mobility does not only involve camels, but also people who look after the camels to provide care along the journey. Import and export of camels have especially increased since the year 2000 (Figure 1C). The imported camels mainly come from the UAE and KSA (Figure 1D).

The dynamics and travel patterns of Qatari camels are complex (Figure 4). Camels are transported to and from different locations, for a variety of purposes, and with a noticeable seasonal pattern. Mobility gets more intensive during the racing and trading season (September to April). Experts believe that the ban of open grazing in Qatar played a key role in the intensity and frequency of camel movements. They mention that there has been a remarkable increase after 2011 in numbers of camel workers and owners who cross the borders to and from KSA along with their animals, although this recently stopped with the KSA-Qatar political situation. The ban of open grazing stimulated camel owners to establish farms in KSA and UAE where open grazing is still permitted. Therefore, camels are moved through Gulf Countries, particularly during the winter season.

Camel races and beauty contests that are routinely organized in nearly all Gulf countries are another factor that boost the national and international movement of camels. Compared to other types of camels, racing camels dominate in terms of numbers and frequency of mobility both across borders and domestically, particularly between September and April. As per the records of the Camel Racing Committee, in the 2016 racing competitions, 14,000 camels from Qatar and camels from the other GCC countries contested [35]. However, owing to the lack of standardized identification system, it was difficult to determine the exact figures and the extent of these movements.

Camels are also being mobilized for reproduction purposes (Figure 4). Mating season (also known as camels' honeymoon) starts in the middle of August and continues through February of the next year, with the high season in the September-October period. Female camels are usually taken from their own location to other farms where selected males are kept particularly for reproduction purposes. About 14,000 female camels are annually being moved for mating. They spend around 1 week at a breeding farm with male camels before they are taken back to their original farms. Programmed mating is exclusively being practiced for race and show camels. The mating season is another seasonal activity that entails intensive movements of camels, camel owners, workers, car drivers and veterinarians.

Figure 4: Seasonality and movements of camels and camel activities. (A) Dark green locations and periods of time indicate a high concentration of camels. The arrows show the direction of camel movements. It is shown that many camels gather and mix at the animal market, racing track, and breeding farms in Qatar in August, September and October. Moreover, there is constant movement to and from grazing grounds and racing tracks outside of Qatar. In March and April, most camels travel back to their barns. (B) shows the seasonality of camel related activities. Most activities take place in the "cold season" from September to April.



Changes in Camel Trade

The Doha wholesale market constitutes the primary hub for camel trading. In parallel with the increased number of camel races, Al Sheehaniya City also grew as a market and has become a hub for trade of racing and beauty show camels in Qatar. The wholesale market in Doha hosts camels and other types of livestock from countries all over the Gulf region. The camels typically stay at the market until they are sold. Camel workers live at the market premises. Camels that are being sold (calves in particular) serve a variety of purposes. They are sold to be slaughtered at the Doha wholesale market abattoir, for breeding purposes, to be trained as racing camel, or to be prepared for camel show competitions.

In recent years, the Doha wholesale market has been surrounded by rapidly growing residential areas. Animals in the market are now in close proximity to the residents. As of 2005, slaughter practices were banned inside residential premises, and can only be performed in official slaughterhouses and exclusively by licensed persons.

Changes in Use of Camel Meat, Milk, and Urine

Camel meat and milk are no longer part of the daily diet of most Qatar inhabitants. Nonetheless, camel meat is a fundamental ingredient of Qatari social events and family celebrations. Production of camel meat and milk has remained stable in the past 30 years. Camel milk is generally kept for personal use, particularly for the perceived therapeutic merits of raw camel milk, as well as camel urine. Experts state that there is an unshakable belief that the regular consumption of camel milk helps to prevent and control diabetes. It is also widely believed in the Qatari community that camel urine and milk can heal skin lesions and other diseases. Camel urine is also regularly used to whiten the skin and face and lighten the hair. The majority of camel owners offer camel milk and urine for free, as a practice of generosity.

DISCUSSION

The role of camels in the transmission of MERS-CoV is well documented [5]. Despite the fact that MERS antibodies have already been detected in camels since 1983 [11] and human contact with animals is not new, human MERS cases were only detected in 2012 [41]. Based on institutional and literature data and in-depth interviews with key professionals, this study sought to examine the changes involving the human, animal, and environmental drivers that may have contributed to the spread and virus spillover to humans.

Our reconstruction of events over the past decades, based on available literature, statistics, and expert opinions, lead to the conclusion that the discovery of oil and natural gas resources has been the starting point of a chain of events that ultimately led to conditions favoring the emergence of MERS-CoV (Figure 5). This discovery led to massive economic growth. Owning a camel represents the wealth and status of its owner in Arabic culture.

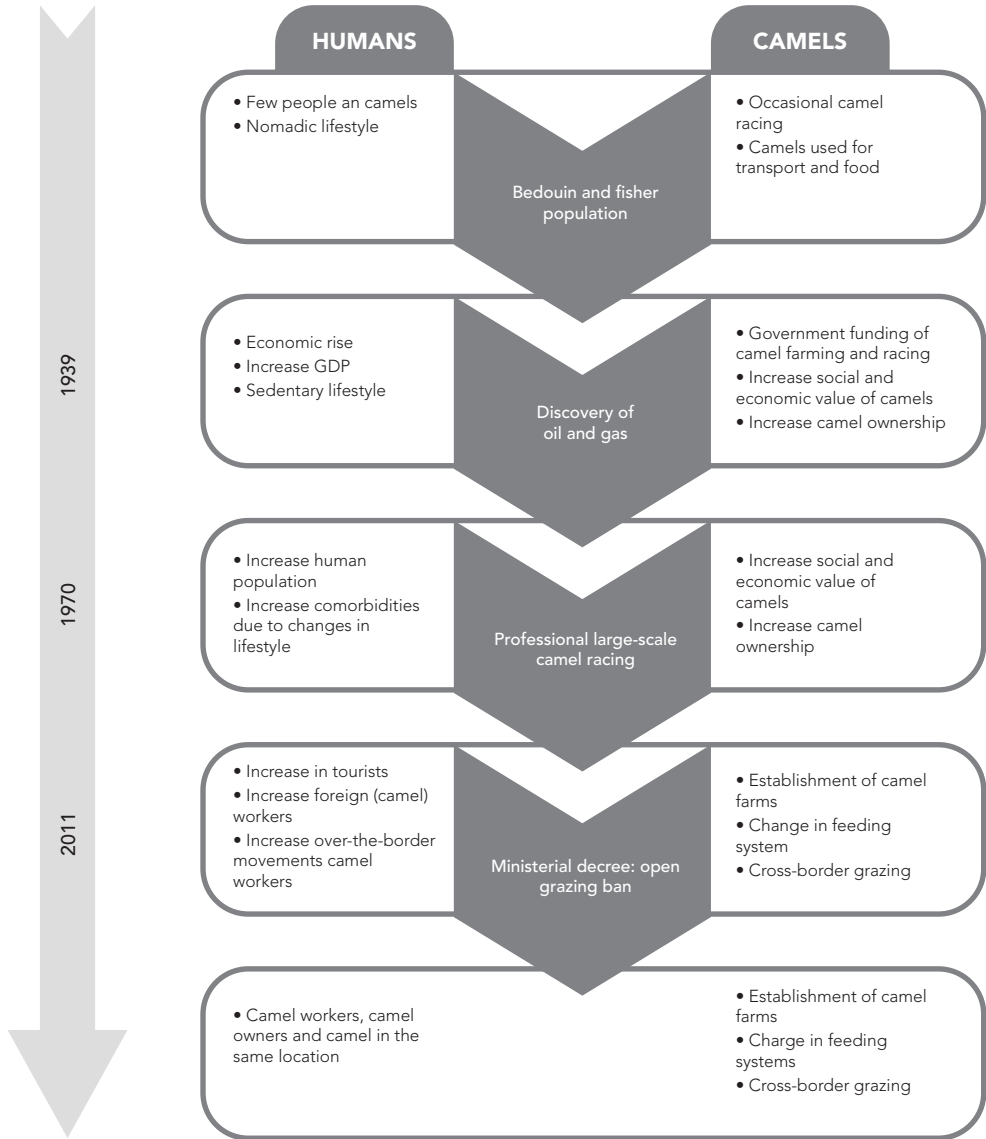


Figure 5: Visual summary/timeline of relevant events.

Governmental sponsorship of camel ownership and camel racing further stimulated the camel industry, especially the camel-racing sector. This in turn led to an accelerating increase of the camel population, a change in camel farming, and a concomitant increase in the number of camel workers [34]. The human population of Qatar has increased by 7-fold over the last decades [23]. This is unlike other high-income countries, that have a yearly overall population growth of only 0.6% [20]. Population growth and high population density have been shown previously to be important risk factors for disease emergence

[42]. Moreover, consistent with the disease profile of wealthy countries where sedentary lifestyle prevails, the prevalence of chronic diseases increased in Qatar in accordance with the increasing GPD, ultimately rendering the Qatar population not only vulnerable to virus transmission, but also to its deadly complications [43].

The intimate nature and number of interactions between camels and humans has also increased significantly in the past 30 years, increasing the risk of any zoonotic spillover. At camel complexes, workers intimately reside, sleep, and eat with their camels. Camel owners, on the other hand, pay regular visits to their barns and stay there for considerable hours every day (even longer during weekends, holidays, and winter season) in the majlis built at the corner of their barns. Owners, who are often of advanced age with multiple comorbidities, enjoy drinking fresh camel milk and entertaining guests. Those who suffer certain diseases tend to visit the camel barns to use camel urine or drink fresh camel milk for its perceived curative properties.

Among the variety of changes that involved camel husbandry in Qatar, the shift from open grazing to close housing systems seems to be most significant. Opportunities for camel-to-camel and camel-to-human spread have greatly increased since then. It is possible that housing camels in barns, with poor biosecurity and hygienic standards, turned these barns into 'melting pots' for the virus that ultimately acquired the ability to cross the human-animal barrier. The increase of cross-border movement of camels increased chances and frequency of (international) virus spread. Camels are transported freely across borders for a variety of purposes through multiple routes and means of transportation. When camels and the humans that accompany them, arrive at the site of a race or beauty event in Qatar, they are housed with the local camels. Owners are welcomed in the majlis at the camel complexes. The mixing of camel and human of different origins further increase chances of virus transmission.

Although much effort was made to study MERS-CoV viral sequences and MERS-CoV transmission between dromedary camels and humans, it is still unknown which genetic mechanisms have caused the viral spillover of dromedary camels to humans. However, the most important determinant of host specificity seems to be the Spike S1 protein, that recognizes and binds to host-cell receptor DPP4 [44]. Recently it has been shown that the MERS-CoV spike can rapidly adapt to species variation in DPP4 [45]. As such, the increasing human-animal interface that is described in this paper may have facilitated the adaptation of the spike protein to human DPP4. However, much remains unknown, also in view of the findings that MERS-CoV from East Africa were not phenotypically different from the viruses from the Middle East, while human MERS patients have not been reported from the African continent [46].

Finally, the changes in animal husbandry practices, earlier weaning, frequent grouping and transportation of animals, and the introduction of an entirely new feeding system, may induce stress in the camels. These changes and movements often involve young weaned animals, at the same time as maternal antibodies are waning, which are linked to the shedding of the virus [42,47]. Most of the limitations of this study were related to

the availability of data. Firstly, statistics on animals, import and export, animal workers, and land use were only found since 2000 onwards, limiting the chance to study the trends and changes prior to that year. Secondly, even the available national data on the animals, humans, and environment were found to be sometimes inconsistent, limiting the possibility to provide “hard evidence” of causality. Nevertheless, this is the first comprehensive quantitative overview of possible drivers of MERS-CoV in Qatar.

CONCLUSIONS

Several key changes were shown to involve camels, humans, the economy, and the environment in Qatar during the last 30 years. Our study indicates that the rapid increase in camel ownership, leading to the presence of camels from different origins in a high-density environment mixed together with human and other animal species may have offered the right circumstances for the virus to spread from camels to humans. The other key changes that were described collectively contributed to this situation. Further understanding of the drivers that led to the emergence of MERS-CoV can serve as input for MERS-CoV surveillance and control measures to prevent further spread of MERS-CoV and reduce transmission from camels to humans.

Supplementary Materials

The following are available online at <http://www.mdpi.com/1999-4915/11/1/22/s1>, Supplementary 1: Categories, subcategories, and data sources used for information gathering in this review; Supplementary 2: Questionnaires used for qualitative information gathering in this review.

Author Contributions

E.F., R.S.S., C.R., and M.K. conceived and developed the conceptual framework; T.V. and R.S.S. executed the literature search; E.F., M.M.I., M.N., and M.A. designed and executed expert interviews; E.F., R.S.S., T.V., and M.K. executed the data analysis; E.F., R.S.S., C.R., T.V., M.N., M.A.T., M.M.I., H.A.R., M.A., F.A., S.A.M., M.A., and M.K. contributed to the interpretation of the results; R.S.S., E.F., M.N., M.M.I., and M.K. wrote the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Conflicts of Interest

All authors declare no conflicts of interest.

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SUPPLEMENTARY MATERIAL

Annex 1: Categories, subcategories, and data sources used for information gathering in this review.

Methodology: This review aims to summarize quantitative dataset containing human, animal, and environmental factors to investigate the possible drivers that contributed to the MERS-CoV emergence in Qatar. The review mainly refers to changes in the last 30 years.

Categories	Sub-categories	Description of sub-categories	Reference	
Human demography and behavior	Population	Total population	1, 2	
		Gender wise population	1	
		Age wise population	1, 3	
		Municipality wise population distribution	32	
		Population by nationality and origin	4, 5, 6	
		Economically active peoples	4, 32	
	Comorbidities and death	Smoking	Smoking	7, 35
			Obesity	8
		Cardiovascular	Cardiovascular	7, 9, 30
			Diabetes	7, 9, 10
		Asthma	11	
		Chronic Lung Disease	9	
		High Blood Pressure	7, 9, 36	
		Kidney Failure	9	
		Chronic Liver Disease	9	
		Chronic Anemia	9	
		Cancer	9	
		Immune Deficiency	12	
		Total death and death rate	9	
		Sanitation	Number of buildings connected to public sewage	Number of buildings connected to public sewage
	Number of buildings connected to water			27
	Access to drinking water		37	
	Population with access to improved water source		13	
Population using improved sanitation facilities	13			
Cultural practices around camels	People living in rural and urban area	18		
	Employment in agriculture	18		
Knowledge level	Educational status of population	14		
	Health education	30		
Economic development	Oil and GDP	16, 17, 18		

Categories	Sub-categories	Description of sub-categories	Reference		
International travel, commerce, sports and leisure	Tourists	Total arrival and number of tourists	15, 18		
		Doha airport arriving passengers	16		
		Origin of visitors	15		
	Camel related travel	Cross border movement and travel of workers and owners	4		
		Camel import and export	19, 23, 24		
		Camel type (Race, show, and others) and origin in import	19		
		Show camel events (number of shows per year)	39		
		Agricultural and food industry change	Camel demography	Total number and density per area	19, 29, 21
			Farming demography	Total number of farms and density per farms	19
			Camel types	Race, show, and others	22, 38
Camel products	Production: milk and meat		22, 40		
Camels slaughtering	Total slaughterhouse and number of slaughtered animals		19, 22		
Feed import	Camel and other livestock		24		
Climate and weather	Other livestock	Sheep, goat, cattle, and horse	40		
	Temperature, humidity and rainfall	25, 26			
	Land use change	surface protected area and cultivable land	27, 28, 40		

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Annex 2: Questionnaires used for qualitative information gathering in this review

Methodology: Interviews with a group of 15 experts and stakeholders from Qatar aimed to summarize quantitative information and remaining data gaps in the quantitative dataset containing human, animal and environmental factors to investigate the possible drivers that contributed to the MERS-CoV emergence in Qatar. The questions mainly refer to changes in the last 30 years.

List of the questions:

1. What are the changes with regard to camel ownership?
2. What are the changes over time with regards to behavior and living conditions of people around camels? Examples are: frequency/intensity of –camel contact, visiting camel’s barns, proportion of people living with camels permanently.
3. What are the changes with regard to cultural habits involving camels, such as kissing camels and uses of camel products (meat, milk, and urine)?
4. What are the changes of the level of educational status of the camel’ workers and owners?
5. What are the changes in the health education activities targeting camel workers and camel owners?
6. What has changed in the cross border movements of camels and people around camels?
7. What are the changes in movements within Qatar of camels and people around camels?
8. What has changed with regard to the demography of camels and camel farms?
9. What are the changes in camel farming practices?
10. What are the changes in the feeding of camels?
11. What are the changes in camel slaughtering practices?
12. What are the environmental changes that took place in Qatar, for example with regard to protected areas (nature conservation) and their effects on camel farming?





Chapter 10

Summarizing discussion

In this study, we sought to review human and animal dynamics to identify factors that might have influenced the emergence, transmission and spread of MERS-CoV in Qatar. In addition, we explored the strengths and challenges faced by the health system in preparing for and responding to the MERS-CoV epidemic. Through this study, a (One Health and Biosecurity) framework is introduced to tackle some of the challenges to control MERS-CoV and other zoonoses at the human-animal interface.

Operationalization of One-Health

Among the viral zoonotic pathogens that threaten humans, MERS-CoV is perceived as a global health security problem in view of continued spill overs and the potential for human to human spread [1,2]. In fact, Leibler et al. [3] argued that most of the zoonotic pathogens threatening human health either arise, or are transferred to, humans from livestock. Therefore, when MERS-CoV appeared, officials from the public health and veterinary side identified a number of questions during the early phase of the epidemic. These questions included: (i) what are the potential reservoirs of the virus? (ii) from where and how do camels contract the infection in the first place? (iii) do camels demonstrate symptoms? (iv) for how long would they shed the virus? (v) what are the circumstances and practices that might increase the risk of the virus transmission to humans? (vi) Who are those at risk more than the others? and (vii) whether such transmission follow particular seasonal trend? These questions, in addition to several others, directed the research work part of which is introduced within this thesis.

Once the early evidence suggested the zoonotic nature of MERS-CoV, the Qatar government embraced the One-Health, multidisciplinary approach with the goal to ensure efficient and coordinated national response to MERS-CoV cases (Introduction, chapter 2 and 6, [4]). Three main strategic actions were taken to establish a One-Health response to outbreaks including MERS-CoV: (1) the development of an integrated National Emergency Preparedness and Response (EPR) Plan. This plan provided an overall guidance to all responders at the national and sub-national levels. Under the umbrella of 'One-Health', the plan was developed with the help from the WHO, OIE and FAO (FAO, 2015); (2) the formulation of a National Outbreak Control Task force (NOCT); and (3) the implementation of joint MERS-CoV investigations.

Outcome of the One-Health Approach

The findings of our studies at the human animal interface in Qatar made an important contribution to the understanding of the potential relationship between humans, animals and MERS-CoV infection. In addition, we have provided evidence for the zoonotic potential of MERS-CoV from camels and the possible risk factors (Chapter 3, 4, 5; [5]). Hitherto, the implemented One Health surveillance helped us to detect twenty-four MERS-CoV cases in Qatar and establish the link with dromedary camels (summarized in Chapter 10). Of these, fourteen cases had direct camel contacts (six camel owners and eight camel workers), and

camels associated with cases tested positive for MERS-CoV RNA and in two cases, virus was identical. These findings have strengthened our knowledge on modes of transmission and the source of infection.

Detection of virus in camels

First definitive proof of camels as potential source for MERS-CoV infection came when the virus was detected and characterized genetically from a nasal swab of camels in Qatar as part of our joint investigations [6]. In the study, the nucleotide sequence of isolated virus from camels swab were matched with MERS-CoV from two persons work in the same farm in Hafr-Al-Batin [7], suggests a recent outbreak incident that involved humans as well as camels. We further confirmed that dromedary camels are a reservoir for MERS-CoV (Chapter 5.2; [5]) and highest rates of virus shedding occurring in young camels (Chapter 5.1; [8]). In addition, as co-circulation of several variants at any given time was detected by strain typing of samples from animals at the central market in Doha, this demonstrated that the high prevalence was probably caused by multiple introductions. This could likely be attributed to the way camels from different origins are grouped together then co-herded for weeks prior to slaughter, providing many opportunities for the virus circulation [9].

Risk factors and at-risk groups

We reported that 80% of the workers from camel slaughter house were positive for IgG antibodies by serological microarray followed by virus neutralization assays, augmenting the perceived evidence of the dromedary camels' role as a source threatened to infect humans. These results also suggest that people with frequent exposure to camel settings could be at higher risk of the infection than healthy adults and/or those with no close contact with camels. This group is not only capable of transmitting MERS-CoV, but also any other similar emerging or re-emerging zoonotic disease. Qatar animal workers are at particular risk in view of the rapid changes in the animal sector over the past decades, described in chapter 4, 9 [9]. Thus, promotion of biosecurity measures and occupational regulations for animal caretakers under the One-Health umbrella is paramount.

These studies contributed to the knowledge needed by the WHO, OIE, and FAO to conclude coherent recommendations to the professionals and the public worldwide [2,10]. All of them were used to update their MERS risk assessment guidelines. Nonetheless, MERS-CoV transmission dynamics within the herd and between dromedaries and humans remained incompletely resolved, providing insufficient evidence to curb the virus spread. Similarly, the events that typically involve intensive human exposure to dromedary camels, like camel racing, have not yet been thoroughly investigated to understand where and how infection occurs at the human animal interface [11].

Amid the lack of conclusive evidence on what caused the virus spillover from camels to humans, public health scholars resort to the belief that the spread of MERS-CoV has likely resulted from the effect of combined factors: the exponential increase in the camel

population roaming the region amid absence of biosecurity precautions coupled with the poor perception of the disease risk among both camel owners and workers (Chapter 4, 9; [12]).

Interventions, policy changes and societal impact

Understandably, separation of naive animals from previously exposed camels and encouraging workers to wear personal protective equipment (PPE) might help decrease the risk of MERS-CoV exposure to humans (Chapter 3, 4, 9; [4]). A national One Health program needs to foster collaboration on training of interdisciplinary teams, risk assessments, surveillance and control measures for MERS-CoV as well as other zoonoses. Regular meetings that joined the two sectors enhanced coordination and the timely sharing of information. Through these meetings decisions makers were advised by public health specialists to allocate the resources needed by the veterinary sector to undertake its role in investigation and response. Formalised and continued cross sectoral collaboration is critical because there is a range of serious zoonotic diseases threats. Sharing the same socioeconomic and climatic conditions, Gulf countries likely are threatened with a number of zoonotic illnesses, further necessitating the promotion of regional collaboration where the one-health can rather become an institutional practice, not a temporary style to respond to an epidemic.

An important benefit of the One-Health collaboration is the change in attitude of the camel owners, who used to deny the joint investigation teams to access their camel barns, but now allow investigation and research teams to examine infection transmission hypothesis along with the risk factors [14]. This in turn most likely led to a reduction in the experiences of both self-stigma and social-stigma associated with MERS-CoV (Chapter 6). In Qatar, the human and animal sector decided to have unified and joint media activities and messages and, as result of that, the local media became more engaged with the authorities and reported more on the effort by the teams to prevent the spread of MERS-CoV. Qatar TV made a documentary reconstructing the outbreak and activities which was broadcasted nationally in 2015/2016. Also, possibly as a result of the raised awareness in the risk group and the general population, patients and families became more co-operative during case investigation, contact tracing, history taking, sample collection and allowed access to their barns and camels. A marked improvement in the adherence to infection control measures among camel owners with co-morbidities was observed and there were less rumors or negative messages circulating in the community about MERS-CoV (Chapter 2, 3, 6).

Challenges faced when implementing the One Health approach

The One-Health approach exposed serious technical challenges involving both the public health as well as the veterinary sector. Different factors can explain these challenges, but having different priorities for the two sectors could be signalled as a prime factor. While

the priorities of the public health sector largely focused on protecting humans from the impact of the epidemics caused by emerging zoonotic agents, the animal and agricultural sector, on the other hand, seems to prioritize health problems that can have direct impact on animal or farm.

Under the pressure to carry out coherent control measures, public health sector had to assume direct responsibility for taking the lead. However, the veterinary sector, as voiced in several 'off-the-record' meetings, seemed to perceive this approach as 'superiority', attenuating the momentum of the joint work. The readiness to perform consistent, coordinated investigation of human as well as animal-suspected cases is one of the key challenges to the joint work including the promptness to initiate field investigation with a designated rapid response team.

Being short of technical staff to undertake the routine as well as the urgent field investigation missions, the veterinary sector had difficulties to keep abreast with the burning needs of outbreak investigations. Moreover, the national capacity to perform advanced diagnostic tests was also lacking. Sequencing of both human and animal specimens is unattainable domestically, limiting the ability to determine whether the source of infection is zoonotic. Similarly, the time needed to obtain governmental consent and arrange the shipping of the specimens abroad to the international reference laboratories has negatively hampered the timeliness of investigation and response. Remarkably, there were no guidelines for the veterinarians on how to investigate camels suspected with such a novel virus and what are the appropriate samples to be collected, how to collect them and when.

Also, since there was no biosecurity system in camel farms, the lack of a camel identification and registration system has negatively affected the joint case investigation; traceability (important given the high mobility of the camels across the international borders and domestically), the likely whereabouts of exposure, and the potential sources of exposure thus were difficult to reconstruct.

Finally, as MERS is not typically considered as serious disease in camels (infected camels typically neither show apparent symptoms nor die because of it), the urge to respond to the reported cases was uneven between the public health and the veterinary sector. The same observation can explain why most of persons at risk continued the practices (like consumption of the unpasteurized milk and the use of camel urine) that can result in them acquiring such a serious infection. Hence, we can understand why the enthusiasm to comply with the biosecurity precautions is almost absent.

Key lessons Learned

One-Health is important to understand a zoonotic disease. The One Health approach has been central to our current understanding of MERS-CoV. Thus, the same approach will have to be maintained to assess the effectiveness of the control measures. As emerging viruses remain to be a constant challenge, One-Health must be fostered at all levels.

Community engagement is paramount: Community engagement substantially helped in undertaking our investigations. A dramatic behavior change was observed among people at-risk who initially denied that camels could be a source for such a disease. During the initial phases of the epidemic, owners of the camel farms denied the joint investigation teams from accessing their farms. These are not true anymore.

Transparency: The constant policy of transparent Emergency Risk Communication allowed the community to be up to date with the situation. It also helped maintain the public trust in the national competent authorities.

Recommendation for scaling up

To upgrade the One-Health approach, a comprehensive system of One-Health surveillance needs to be established to ensure the early reporting and joint investigation of suspected human and animal cases along with contact tracing. The elements of this system should include the following components: Severe Acute Respiratory Infections (SARI) surveillance; active surveillance of people-at-risk for MERS-CoV infection (such as persons around camels); and testing of camels at slaughterhouses, camel race and at the ports of entry of Qatar. Additionally, effective joint training programs are needed for veterinarians and physicians to ensure the early detection of suspected human and animal cases. Moreover, to increase knowledge on the prevalence and epidemiology of MERS-CoV, a large-scale case-control study along with a sero-epidemiological survey targeting the most-at-risk groups needs to be designed.

Camel farm biosecurity

As a system for management, the surveillance should be complemented by a farm biosecurity system, which is supposed to limit the virus transmission in camel populations (through development of vaccines and effective management of infected animals/herds) and thereby reduce the opportunity for further human exposure. It is well established that infectious diseases that spread from animals to humans, such as salmonellosis, cryptosporidiosis, rabies and many others have been reported in veterinary personnel [16-23]. It is argued that such vulnerability could be substantially lowered by applying evidence-based precautions like biosecurity protocols and infection prevention and control measures at the animal care settings and biosafety measures at the healthcare facilities respectively. There is no doubt that ignoring such preventive actions increases the chance for serious outbreaks to spread.

We have previously shown that the risk to acquire MERS is proportionate to the exposure to camel farms where biosecurity measures and hygienic practices are largely ignored (Chapter 2, 3, 9; [8,9]). One of the serious observations reflected in the drivers for MERS was the intensive, poorly traceable movement of camels along with their caregivers across the borders and domestically between wide range of entertainment, trading

markets, abattoir house, and husbandry farm settings. Another observation was the frequent mixing of camels from various origins and the level of direct contact with humans, some of whom suffer chronic illnesses, rendering them susceptible to MERS. When reading this with the prevailing low risk perception among both the camel owners and workers, as described in anthropological studies [14], the vulnerability to the risk of the disease multiplies. Nonetheless, such vulnerability could significantly be abated if the biosecurity simple measures were applied.

As a result of the drastic sociopolitical and economic change experienced in Qatar in recent years (Chapter 9), novel routes from importation of food were established along with powerful encouragement to produce food, including from animal resources, domestically. These developments are likely to exacerbate the already existing risks of zoonotic diseases precipitated by the lack of biosecurity, poor hygiene, the low risk perception, and the constant vulnerability to occupational hazards. As the number of farms endorsed for agriculture and animal production is rapidly mounting, it is thus preferable to promptly embrace biosecurity measures along with the other preventive strategies rather than regretting an avoidable waste of precious lives and livestock.

Biosecurity is defined as “the suite of good management practices that are implemented on farms to reduce the entering, spreading, or establishment of disease agents, which further reduces risks to the environment, community, and the economy” [27]. In fact, biosecurity is deemed the best-known effective way to prevent the introduction of MERS-CoV and other zoonotic diseases into camel farms. Recently, it has been argued that biosecurity is the most economical and effective method of zoonotic diseases’ prevention and control, particularly when coupled with educating and training the staff in the farms [24,25].

The objectives of such a system should be to (1) ensure early detection of MERS-CoV in the camel production, (2) to implement proportional and sustainable measures to limit the spread of the virus in the camel population, and (3) to limit occupational exposure of personnel to the virus.

Development and implementation of the camel farm biosecurity plan

Undoubtedly, the effective implementation of biosecurity largely depends on good planning and resources. Biosecurity calls for sophisticated culture of safety and hygiene to be performed by caretakers of all types of farms/barns. Several steps need to be taken under the technical guidance of experts in the field to develop and implement the biosecurity plan on a farm. Defining the program objectives, developing tools for risk assessment, assessing occupational health vulnerability, along with the practices that influence environmental hygiene. Further, the plan must give detailed guidance in terms of standard operating procedures (SOPs), staff training, and monitoring the effectiveness of the biosecurity plan. Through these steps, risks and hazards can be classified and prioritized. However, to ensure the appropriateness and feasibility of the plan, it is fundamental to involve the relevant stakeholders and define how they perceive the risks to which they might be exposed.

The role of these stakeholders would not be confined to identifying the social and cultural acceptability of the proposed measures, but also, they can noticeably facilitate the foundation step of assessing the risks. Such step can draw from the technical risk assessments which explains the capability of an infectious agent to spread across different farms in a vast geographic location. Moreover, the stakeholders can help and provide reasonable understanding of the community motivations, calculate the estimated cost for the required items and whether it would be affordable for the community to bear it.

However, a more potent way of showing the seriousness of the biological hazards is to use the same mechanism to engage the target stakeholders in reflecting the socioeconomic effects of a natural-occurring outbreaks among animals. Events as such create an ideal chance to explain threats, how they cause harm, and the ways to prevent them along with their avoidable consequences. Therefore, vigilant animal surveillance to detect epidemics, combined with professional investigations that include potential zoonotic threats can persuade the target community members with the credibility of the authority's recommendations. Ensuring the preparedness to manage such events will contribute to public trust on the value of the biosecurity measures.

Biosecurity in its essence calls for a drastic behavioral change in the current organization of camel farming, investing in a package of incentives, penalties, and regulations. Adoption of a biosecurity system will not only require a burden of practices that are unfamiliar to many of those involved in farming business, but also will add a substantial cost on them. Therefore, it might need to be marketed as an essential value to preserve the wellbeing and prosperity for the individuals, their families, and the entire community. A blended approach of carefully designed risk communication, behavioral change, and health promotion strategies can help achieve the desired effect. Surely, these strategies are not cheap and on top of that, it will take some time to exhibit tangible results.

Approaches to implement biosecurity might differ according to the farm type and size. For instance, regulatory authorities can use their power in controlling the access of farm products to the markets as an incentive to promote the abidance to biosecurity measures across large-scale farms with commercial production. Whereas in small-scale farms, biosecurity can rely on establishing physical barriers to control access. However, such barriers, along with the other in-kind materials and consumables, should be part of the project budget.

A risk-based pilot study can help identify the critical control points, prioritize the biosecurity interventional measures, and assess the support from the farm owners and workers, putting into account motives with which they shall abide to.

Where to install the biosecurity measures?

The risk assessment during the preparatory and pilot phases of the program is anticipated to yield a prioritized list of high-risk settings. For MERS, camel farms particularly in the main hubs for race competitions in Shahanyia and the trade in the wholesale market will

be considered. Additionally, as noted in studies conducted on MERS-CoV in Egypt [26], quarantine areas are likely to be one of the critical points where the virus transmission can occur when imported camels mix with each other for several days before they are redeployed inside the country. In addition, although it might rather fall under the jurisdiction of occupational health, abattoir and veterinary clinics shall firmly be included.

Components for the camel farming biosecurity system

The basic principle elements of farm biosecurity are segregation, cleaning and disinfection. Infected animal shall be denied access to mix with uninfected animals. Likewise, vehicles, equipment, and materials should all be cleaned before they are allowed into the farm [27]. The components of the system include: (i) the farm location, design, and management. It also includes a system that operates according to national and international guidelines for farm biosecurity. It guides how farms shall be categorized, licensed, along with the routine farm inspection and notification of events; (ii) control of animal movement to and from the farm as animals frequently taken out for variety of entertainment and business purposes; (iii) the protocol to manage importation of new animals, screening process, and isolation of both sick and new animal [28]. Additionally, the husbandry, which is rather an occupational field, will also deal with the animal welfare. Some of the activities include: health and safety training, registration of workers, annual health check-up, timely vaccination of caretakers, the process of camels' raising, breeding, and feeding [28].

Health management of animals

As shown in this thesis active circulation of MERS-CoV was found in animals when transported and grouped in high risk areas such as camel racing and show competition, slaughter house, camel market (Chapter 3, 4.1, 8, 9). Therefore, a framework was proposed. This framework includes: active surveillance covering farms, quarantine stations and other similar settings. Whenever an animal tests positive by PCR at one of the designated screening visits, the following response actions should be taken regardless of epidemiological link to human case: i) immediate notification to animal health authorities and the public health sector; ii) both sectors should carry out rapid joint animal-human investigation with retrospective or prospective tracing for the animal movement using standardized investigation forms. The entire herd should be screened besides reviewing the history of contacts of the persons linked to the infected animal; iii) if the investigation detected confirmed human cases suspected of being community-acquired, veterinary authorities must be informed; iv) the interpretation of epidemiological and laboratory data should be done along with sequencing of viruses from both animals and human cases; v) the outcome of such investigation should be communicated with the OIE as well as the public in accordance with the emergency risk communication guidelines; vi) the veterinary team must undertake control measures as part of the field investigation. As a precaution measure, isolation of infected camels shall continue until PCR testing turns negative. Milking and slaughter of positive camels

supplying the food chain must be banned throughout the period of quarantine. All persons in contact with infected animal must put on the appropriate protective equipment.

Preventive interventions for visitors and other at-risk groups

When affected with MERS-CoV, dromedaries do not display any signs of infection [29-35]. Therefore, it is unlikely to identify all animals on a farm, marketplace, racetrack or abattoir that are shedding the MERS-CoV, potentially exposing humans to the infection. However, infected camels may shed the virus through nasal and eye discharge, faeces, and possibly in their milk and urine [36-39]. The virus may also be found in the organs and meat of an infected camels [12,40]. Several conducted studies suggested that visitors to the camel barns contracted MERS infection [41,42].

Based on the literature who approach camels' and their setting apart from the veterinarians, we found that camel farm owners and workers in addition to the visitors were exposed at varying degrees to the camels over a course of different seasons. Animal workers, who are often young and healthy, experience a highly intensive, frequent, and intimate exposure to camels, but only few of them contracted the disease, and displayed clinical outcome (Chapter 2, 3, 4.1 and 9). However, it is unclear if that implies that they are less likely to be transmitting virus when infected. Camel owners, who were predominantly middle-aged with chronic illnesses and reported a less frequent exposure to camels are more vulnerable for developing severe disease [31,41,43-47]. As farms also serve a social function, visitors to the camel farms also can arguably have a similar degree of vulnerability to the owners. Therefore, they can both be put in the same category for the guidelines of biosecurity measures [48,49].

Anthropological studies [14] revealed several social norms imply exposure to MERS infection. Camel owners spend their weekends in their 'Majlis' where they receive visitors and guests. A constant practice of entertaining guests is offering the fresh camel milk with dates and coffee to display the Arabic generosity. It is worth noting here that MERS-CoV was detected in raw milk of infected dromedaries (Chapters 2, 3, 4, 5 and 9; [6,8,40]). Until more is understood about MERS, males above 60 years of age with comorbidities such as diabetes, renal failure, hypertension, chronic lung disease, obesity, and low immunity should avoid direct contact with dromedaries with unknown infection status, and abstain from drinking or eating raw animal products in the first place.

Furthermore, drinking raw milk or consuming undercooked camel products, including meat, blood and urine, implies a high risk of infection from a variety of other organisms that might cause disease in humans. Among others, relevant for Qatar are *Brucella abortus* and *Brucella melitensis*. When animal products are appropriately processed, they will likely become safe for human consumption. Yet, other hygiene practices should persistently be observed to prevent cross-contamination [50,51].

As a general safeguard, anybody visiting farms, animal marketplaces, barns or other places where dromedaries are present must practice the universal hygiene measures;

consistent hand washing after touching animals, avoiding touching eyes, nose or mouth with hands, and avoiding contact with sick dromedaries. It is no less important to wear protective gowns and gloves while professionally handling animals. Such recommendations should also be disseminated to travellers, tourists and pilgrims coming to the region from around the world [10].

Workers in dromedary farm, abattoir, animal market, veterinary personnel and those handling camels at racing farms should all be instructed to comply with hygiene and biosecurity standards, including frequent hand washing after touching animals and using PPEs. Workers should also avoid exposing household members to dirty work clothing, shoes, or other contaminated items that may have come into contact with camel excretions and fomites. It is therefore highly recommended that these clothes and items remain at the workplace to be washed daily, and that workers to have access to wash facilities at their workstations before leaving the farm. The biosecurity guidelines shall give more details on how to change cloths and skills of hygienic practices [50].

Wisely, infected animals should never be slaughtered for feeding purposes; deceased animals should be safely buried or burned. If not protected, people should abandon contact with animals tested positive for MERS-CoV until consequent tests reveal that the camel is free from the virus or of other public health threats [51].

Vaccines

Vaccination is one of the most practical proactive measures, which significantly prevents infection, replication and shedding of the pathogen while ensuring sustained animal production. Vaccines are increasingly used in animals for variety of purposes. Primarily, vaccines are used to improve animal health and consequently animal production, by managing infections and infestations among animals. In addition, vaccines can be used to indirectly protect the public health by administering vaccines that can prevent or curb infections and shedding of pathogens from animals, particularly emerging or re-emerging zoonotic ones [52]. Therefore, inoculation of camels can be considered a risk mitigation option as well as a biosecurity intervention directed to target the human-animal interface to substantially reduce MERS-CoV spillover to human population [53-56].

Equivac[®], a Hendra virus vaccine can be taken as a good example for such a good intervention. Equivac was developed solely for horses as a precedent for mitigating the risk of zoonotic disease among humans with a vaccine administered to animals. However, the difference for MERS CoV is that a camel vaccination in this context would be deployed solely to protect humans, as the virus causes only mild upper respiratory illness in camels, unlike Hendra virus which causes severe disease in horses as well. It is for scholars in public health, veterinary sector and pharmaceutical manufacturers to tell whether this method can be replicated to managing the risk of MERS-CoV. A similar proposed preventive strategy that needs to be assessed is the production of human vaccine against MERS-CoV to offer long term protection for those with high vulnerability risk factors. Such human vaccine might also prove valuable during prospect MERS outbreaks [55,57-61].

Currently, there are no MERS-CoV-licensed human vaccines as the process leading to its production is not yet at industrial scale. However, a number of human vaccine candidates for coronaviruses, including MERS-CoV, are at various phases of development. Reports tell that five overall vaccine technology platforms targeting the MERS-CoV spike protein have been processed to develop the inoculation [55]. Fortunately, a number of countries and public health agencies called upon pharmaceutical companies to urge these companies accelerate the development of a dromedary camel vaccine in order to evaluate the potential to limit the chances for the virus transmission to humans [55,62].

Operationalizing the vaccination

Ahead of the mass production and deployment of a dromedary camel vaccine, several critical aspects essential to be evaluated; the vaccine acceptability, cost-effectiveness, and feasibility of its administration in comparison with the other typical interventions. A technical advice is desperately needed to tell which is better: to go for the production of dromedary camel vaccine, a combined camel vaccine that includes protection from diseases that are relevant for the camel production sector, or a human one, provided the formerly mentioned points. Prior to administering the camel vaccine, camel owners and the competent governmental agencies have to be consulted. Additionally, feasibility studies is an essential preparatory part, as it can help explore opportunities for commercial manufacturing and characterize incentives for camel vaccination. Further, the careful assessment of the potential implications on trade is no less important.

Conclusion and main recommendations

The studies described in this thesis helped improve our understanding of MERS-CoV, our ability to identify, early detect, and respond to infection in camels as well as humans, the way we communicate our results and how we use evidence to inform policy decisions to protect camels and prevent new human infections. The results provided compelling evidence that: i) the novel corona virus is circulating in camel herds; ii) camel workers, and camel owners, are at high risk of exposure to MERS-CoV; and iii) abattoirs, camel market, and camel racing areas are high risk settings for MERS-CoV exposure. Despite the progress in our understanding of MERS-CoV, however, many questions remain unanswered. The definitive origin, particular mechanism of transmission, and the factors explaining the seasonal variability of the infections at the human-animal interface are some of the areas that need further research efforts. Determining the principal route(s) of the virus transmission between camels and humans is fundamental to preventing the spread of MERS-CoV into human populations.

MERS-CoV persists to be a significant concern to public health, including potential for adaptation resulting in more efficient human to human transmission. The high case fatality rate underscores the need to expedite well-designed clinical trials for direct, effective therapies and vaccines to reduce the forthcoming economic and public health impacts of

MERS-CoV. Given the documented camel role in MERS-CoV transmission, the common practices in the affected region, like consuming unpasteurized camels' milk and lack of hygiene practice at farms, should be discouraged.

There are systematic challenges impeding the effective embracement of the One-Health in terms of preparedness, investigation and response to MERS-CoV. As new infectious agents will continue to emerge at the human-animal interface, operationalization of the One-Health is the only strategic solution, and prevention and preparedness programs, including farm biosecurity and the One-Health surveillance, investigation, and response, are paramount. Additionally, guidance for surveillance in animal population, quarantine procedures, management of dromedary camels actively shedding the MERS-CoV, food and environmental safety practices at farms, and biosecurity measures at the camel markets, racing yards need to be developed or updated.

While many research efforts were conducted on MERS-CoV over the past seven years, the future focus should aim to generate evidence for public health policies, strategies and interventions. This research agenda should address the following technical priority areas: epidemiology and transmission among both human and animal populations; virus origin and its genetic characterisation; clinical management of cases besides infection prevention and control at the healthcare facilities; treatment and vaccine development and implementation; and impact of prevention interventions and the operational research.

The experience gained out of these studies also provides an opportunity to extend the work on MERS to other zoonoses, including viral, bacterial and parasitic pathogens, of public health significance, as several of the observed findings also may apply to other pathogens. A national zoonotic diseases control program needs to be proposed, including the development of a zoonotic diseases control operational framework, involving different organizations with various scientific and professional backgrounds. They should actively cooperate and collaborate on training of interdisciplinary teams, risk assessments, surveillance and control measures of MERS-CoV, other zoonoses and implementation of joint field investigations

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Appendices

Summary of the thesis

Arabic summary

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Summary of the thesis

The reporting of human cases of MERS-CoV in Qatar during 2012 has sparked intensive investigative efforts to identify factors that might have influenced the emergence, transmission and spread of MERS-CoV. A “One Health” approach was found to be the most feasible and practical way to study the human and animal dynamics in relation to MERS-CoV. Recognizing interconnection of human and veterinary health, “One Health” uses a multidisciplinary and cross-sectorial approach to address (re)emerging risks that originate at the animal-human-ecosystems interface. We explored the strengths and challenges faced by health system partners in preparing for and responding to the MERS-CoV epidemic. The objective of this thesis was to gain knowledge on the ecology and epidemiology of MERS-CoV in Qatar and to enable evidence-based prevention and intervention strategies to reduce the risk of infections at human-animal interface. Additionally, it reviews the current epidemiology and clinical presentation of MERS-CoV infection while describing the preparedness plans to combat the disease.

A comprehensive literature review was conducted in order to determine the gaps in knowledge about MERS-CoV infection at human-animal interface and to lay the foundation for the overall research studies conducted in this thesis (Chapter 1). To better understand the emergence and modes of transmission of MERS-CoV infection in Qatar, a descriptive epidemiological investigation of MERS human cases in Qatar was conducted (Chapter 2).

Camel workers were found seropositive for viral antibody in West Qatar (Chapter 3). The case-control study showed that participants who were found seropositive were more involved in camel training and herding, cleaning farm equipment, or milking camels. In addition, seropositive workers were less likely to wash their hands before and after animal contact and were more likely to handle camels that travelled abroad. Contact with camel excretions and subsequent touching of mucous membranes was likely an important source of infection. The identified risk factors can be used to establish infection prevention and control measures for MERS-CoV by introducing farm biosecurity system. (Chapter 3).

With the objective to design prevention measures as well as explaining the reportedly high MERS-CoV mortality rate, a population-based serosurvey was conducted among camel-contact versus non-camel contact personnel (Chapter 4). Serum-neutralizing antibodies were only detected among camel-contact persons of the 498 randomly sampled sera, suggesting exposure to dromedary as a major risk factor for the infection. Further, upon studying exposure risk factors for MERS-CoV, a high risk was shown by not using personal protection equipment for workers at Doha slaughterhouse (Chapter 4). When investigating MERS-CoV shedding patterns, a high proportion of camels presented for slaughter in Qatar showed significant evidence for nasal MERS-CoV shedding compared to both fecal and oral shedding. This led to the conclusion that nasal swabs constitute

the samples of choice for diagnosis and surveillance of MERS-CoV in camels (Chapter 5.2). Sequence analysis showed that at least five different virus strains were found to be circulating in Qatar, suggesting the slaughterhouse in Doha as a driver of MERS-CoV perpetuation as well as a high-risk area for human exposure. As no correlation was observed between RNA loads and the levels of neutralizing antibodies among examined camels, it was inferred that the immune protection is limited and the potential for reinfection is likely regardless previous exposure (Chapter 4 and Chapter 5.1). After MERS-CoV was first isolated from camels, the phylogenetic analysis of the complete genome clearly showed that MERS-CoV camel/Qatar_2_2014 was very similar to human MERS-CoV. It was also the closest relative to MERS-CoV England/Qatar1 2012 (Chapter 5.2). These data supported the hypothesis that dromedary camels are a reservoir for MERS-CoV and can transmit the infection to humans. The evidence for a possible role of food-borne of MERS-CoV infection was investigated. Camel milk and urine were collected from the high risk areas. We found MERS-CoV specific antibodies in urine samples while all camels showed such evidence for a (previous) MERS-CoV infection in serum (Chapter 5.3). Raw milk samples were tested for anti-MERS-CoV antibodies using both protein microarray assay and virus neutralization with parallel testing of serum, nasal and rectal swabs for multiple genomic targets. All sera and milk samples were positive for MERS-CoV antibodies. Moreover, the presence of MERS-CoV RNA in milk of camels which actively shed the virus warrants measures to prevent putative food borne transmission of MERS-CoV (Chapter 5.4).

A case study describes how One Health approach was initiated and used to develop and establish surveillance and response to MERS-CoV in Qatar during 2012 (Chapter 6). Initial emergency response actions were identified through the Qatar national outbreak control task force including a joint national human-animal health investigation team. Requesting inputs from several international organizations, a comprehensive roadmap for MERS-CoV surveillance and response on the human-animal interface for Qatar was generated. Research findings were used to provide national and international guidance for studies and prevention measures. A survey was conducted among the governmental health and veterinary authorities to monitor preparedness and response to MERS-CoV epidemic through 'One-Health' approach (Chapter 7). Nominating lack of political will as one of the key gaps to adopt 'One-Health', that was also mentioned in 'Doha Declaration' to take the epidemic as a chance to promote the inter-sectorial collaboration to contain MERS-CoV epidemic and to enhance preparedness to combat other possible future emerging zoonotic diseases.

To provide an overview of current knowledge on the distribution, spread and risk factors of infections in dromedary camels, a systematic review was carried out where published data MERS-CoV was compiled and analysed. (Chapter 8). Camels only show minor clinical signs of disease after being infected with MERS-CoV. Serological evidence of MERS-CoV in camels has been found in 20 countries, with molecular evidence for virus circulation in 13 countries. The seroprevalence of MERS-CoV antibodies increases with age in camels,

while the prevalence of viral shedding MERS-CoV RNA detection in nasal swabs decreases as determined by. In several studies, camels that were sampled at animal markets or quarantine facilities were seropositive compared to those at farms as well as imported camels vs. locally bred camels. Some studies show a relatively higher seroprevalence and viral detection during the cooler winter months. Knowledge of the animal reservoir of MERS-CoV is essential to develop intervention and control measures to prevent human infections (Chapter 8).

To identify and quantify key possible drivers that might have contributed to the MERS-CoV emergence and spread in Qatar (Chapter 9), a list of potential human, animal and environmental drivers for disease emergence were identified utilizing literature review, database analysis, and expert opinions. Observing that the discovery and subsequent exploitation of oil and gas has led to a fivefold increase of Qatar GDP and a seven-fold increase in the population in the past 30 years, the resulting increase in income has gradually transformed lifestyle from Bedouin life to urban sedentary life. The subsequent flourishing of the governmental-supported culturally embedded camel sector has led as early as 1990 to duplication of the camel numbers. Experiencing overgrazing and desertification, open grazing was banned in 2005. Replacing this with compact barn housing, camels, camel attendants and other animal species were forced to live in contact and significantly cross borders to seek new grazing areas. Such major habitual changes might have offered the virus the right circumstance to spill over from camels to humans and spread throughout Northern Africa and the Middle East (Chapter 9).

An integrated framework for One Health and camel farm Biosecurity was proposed as a practical strategy to prevent and control MERS-CoV and other zoonoses at the human-animal interface (Chapter 10).

Arabic summary

أول حالة تسجل للمرض بين البشر كانت عام 2012 إلا أن الفيروس المسبب لمتلازمة الشرق الأوسط التنفسية (كورونا) رصد في عينات أخذت من الجمال منذ ثمانينيات القرن الماضي. طرأت تغيرات هائلة خلال هذه العقود الثلاث، بعضها مهد الظروف لينتقل الفيروس من الحيوانات للبشر في قطر وبقية دول الخليج العربي. وقد أشعل تسجيل إصابات بين البشر بفيروس كورونا خلال عام 2012 انطلاقة جهود استقصائية مكثفة في قطر ترمي لتحديد العوامل التي يحتمل تأثيرها على ظهور فيروس متلازمة الكورونا التنفسية وانتقاله وسريانه. وقد برز نهج "الصحة الواحدة" One Health كأفضل مقاربة يمكن تبنيها بفضل جدواها العملية لدراسة حركة الإنسان والحيوان ومدى ارتباطها بمتلازمة فيروس الكورونا الجديد. وفي سياق الإقرار بالارتباط الوثيق بين صحة البشر والصحة البيطرية، جمع نهج الصحة الواحدة مختلف التخصصات عبر العديد من القطاعات بهدف معالجة المخاطر سواء المستجدة أو الناشئة التي تصدر عن التداخل بين النظم الإيكولوجية الحيوانية – البشرية. وقد عملنا على استكشاف مواطن القوة والتحديات التي تواجه شركاء النظام الصحي في سبيل التأهب والاستجابة لوباء متلازمة فيروس الكورونا الجديد.

وقد هدفت هذه الدراسة للتعرف على الخصائص الوبائية والإيكولوجية لفيروس الكورونا المسبب لمتلازمة الشرق الأوسط التنفسية في قطراتخاذ التدابير الوقائية والاستراتيجيات المبنية على الأدلة العلمية للحد من مخاطر سريان العدوى في معرض التداخل بين الإنسان والحيوان. كما تستعرض هذه الأطروحة المظاهر الوبائية والأعراض السريرية الحالية لعدوى لفيروس الكورونا مع وصف خطط التأهب لمكافحة المرض.

وقد أجري استعراض شامل لما نشر بغرض تحديد الفجوات في المعرفة بالعدوى بالفيروس في معرض التداخل بين الإنسان والحيوان وكذلك لوضع أرضية تمهد للدراسات البحثية ضمن هذه الأطروحة (الفصل الأول). من أجل فهم طبيعة نشوء فيروس الكورونا وأنماط سريانه في قطر، أجري استقصاء وبائي وصفي شمل جميع الحالات البشرية التي رصدت إصابتها (الفصل الثاني).

وقد وُجد أن عمال تربية الإبل في غرب قطر تحتوي أمصالهم أجساماً مضادة لفيروس الكورونا (الفصل الثالث). كما أظهرت دراسة شملت الحالات والشواهد (case-control studies) أن المشاركين الذين كانت نتائج فحوصاتهم المصلية إيجابية للأجسام المضادة كانوا أكثر انخراطاً في أنشطة تدريب الإبل ورعايتها وحلبها أو يقومون بتنظيف المزرعة والمعدات الموجودة بها. كما وُجد

أن الأشخاص الإيجابيين للأجسام المضادة لفيروس كورونا أقل اعتناءً بغسل الأيدي سواءً قبل أو بعد التعامل مع الإبل، أو كانت فرصة ملامستهم للإبل المرتحلة عبر الحدود أكبر.

كما لوحظ أن ملامسة إفرازات الإبل وأغشيتها المخاطية كان من أبرز المصادر المحملة لاكتساب العدوى. ويمكن الاستفادة من عوامل الخطورة التي جرى تحديدها لتأسيس تدابير الوقاية ومكافحة العدوى بفيروس كورونا بالشروع في تفعيل نظام الأمان الحيوي في المزارع (الفصل الثالث).

وفي إطار تطوير التدابير الوقائية وتفسير معدلات الوفيات المرتفعة لحالات فيروس كورونا، أجريت مسوحات مصلية بين السكان لتقدم مقارنة بين أولئك الذين هم على تماس مباشر مع الإبل في مقابل غيرهم (الفصل الرابع). وقد رصدت الأجسام المضادة المعادلة لفيروس كورونا فقط من الأمصال المستخلصة ممن هم على اتصال بالإبل ضمن عينة عشوائية ضمت 498 عينة شخصاً ما يشير إلى أن التعرض للإبل أحد عوامل الخطورة للإصابة بالعدوى. وعند التحقيق في مخاطر التعرض لفيروس كورونا، وجد خطر الإصابة كان عالياً لدى غير المستخدمين وسائل الحماية الشخصية من قبل العاملين بمسلخ الدوحة المركزي (الفصل الرابع).

وعند التحقيق في أنماط إفراز فيروس كورونا، وجد أن نسبة عالية من الإبل التي تقدم للذبح أظهرت أدلة معتبرة على ذرف الإبل للفيروس من الأنف أكثر مما تذرفه عبر البراز أو الفم. أدى ذلك إلى استنتاج أن مسحات الأنف هي العينات المفضلة لأغراض تشخيص وترصد فيروس كورونا في الإبل (الفصل الخامس 5.2)

وبجانب ذلك أظهر التحليل أن ما لا يقل عن خمس سلالات فيروسية مختلفة تسري في هذه الأماكن، مما يشير إلى أن المسلخ المركزي في الدوحة يمثل أحد محركات استمرار العدوى بالفيروس وأنه منطقة يزداد فيها خطر تعرض البشر للإصابة بالعدوى.

وتشير ملاحظة عدم وجود علاقة بين كميات الحمض النووي الريبي RNA ومستويات الأجسام المضادة المعادلة إلى احتمال أن المناعة التي تكتسب من التعرض السابق للفيروس تبقى محدودة ما يترك المجال مفتوحاً لاحتمال الإصابة بالعدوى مجدداً بصرف النظر عن التعرض السابق (الفصل الرابع والخامس 5.1)

بعد الكشف عن عزل فيروس كورونا لأول مرة من الإبل أظهر التحليل الكامل لجينوم الفيروس أن سلالة الفيروس الذي جرى عزله من الإبل في قطر MERS-CoV camel/Qatar_2_2014 كان شديد الشبه بنظيره الذي يصيب البشر كما كان الأقرب بالنسبة لسلالة الفيروس الذي جرى عزله من المريض القطري في بريطانيا England/Qatar_1_2012. إن هذه البيانات تدعم فرضية أن الإبل هي مستودع فيروس كورونا وأن الإبل بإمكانها نقل الإصابة للبشر.

وللتحقق من الأدلة في وجود دور محتمل للغذاء في نقل العدوى بفيروس كورونا، جُمعت عينات من حليب الإبل وأبوالها من المناطق عالية الاختطار. وقد وجدنا الأجسام المضادة الخاصة بفيروس كورونا في عينات البول، حيث أظهرت العينات المصلية لجميع الإبل حصول إصابة سابقة. كما اختُبر احتواء عينات من حليب الإبل الخام لأجسام مضادة للفيروس باستخدام تقنية مقايضة بروتين ميكرو آرراي (Protein microarray assay) وتحييد الفيروس مع اختيار مقايضة مصلية مواز لمسحات الأنف والمستقيم (الفصل الخامس). وقد جاءت جميع عينات الأمصال والحليب إيجابيةً بالنسبة للأجسام المضادة لفيروس كورونا. إن اكتشاف وجود إفراز من أنف و / أو براز الحيوانات التي لديها مستويات عالية من الأجسام المضادة المحايدة يشير إلى وجود مناعة غير وقائية. علاوة على ذلك، فإن وجود الحمض النووي الريبي لفيروس كورونا MERS-CoV RNA في حليب الإبل التي تفرز أو تدرج الفيروس بشكل فعال يستدعي اتخاذ تدابير لمنع انتقال الفيروس المزعوم عن طريق الأغذية (الفصل الخامس 5.4).

أجريت دراسة حالة لتصف كيفية أعمال نهج الصحة الواحدة لوضع وتأسيس أنشطة لترصد فيروس متلازمة كورونا التنفسية والاستجابة لها خلال عام 2012 (الفصل السادس). تم تحديد الإجراءات الأولية للاستجابة لحالات الطوارئ من خلال فريق العمل الوطني لمكافحة الأوبئة في قطر والذي ضم فريق استقصاء مشتركاً لصحة الحيوان والإنسان. واستناداً إلى مدخلات طُلب من العديد من المنظمات الدولية أن تقدمها، وُضعت خارطة طريق شاملة لترصد فيروس كورونا والاستجابة له في معرض الواجهة بين الإنسان والحيوان. وقد استخدمت نتائج البحوث لتوجيه تدابير الوقاية وكذلك الدراسات العلمية ذات الصلة على المستوى الوطني والدولي.

أجري مسح استهدف السلطات الصحية والبيطرية الحكومية لتقييم التأهب والاستجابة لوباء متلازمة كورونا استناداً إلى نهج الصحة الواحدة (الفصل السابع)

وعلى اعتبار أن ضعف الإرادة السياسية مثل أحد أبرز الثغرات الرئيسية التي تحول دون تبني نهج "الصحة الواحدة"، ركز "إعلان الدوحة" على هذه القضية بغرض اتخاذ الوباء كفرصة لتعزيز التعاون بين القطاعات وصولاً لاحتواء وباء فيروس كورونا وفي نفس الوقت تقوية التأهب لمكافحة الأمراض المشتركة الناشئة التي يحتمل ظهورها مستقبلاً.

لتقديم نظرة عامة حول مدى الإلمام الحالي بتوزيع وانتشار وعوامل الخطر للعدوى في الإبل، تم إجراء مراجعة منهجية تم من خلالها تجميع البيانات المنشورة حول فيروس كورونا وتحليلها (الفصل الثامن). تُظهر الإبل فقط علامات سريرية بسيطة للمرض بعد إصابتها بفيروس كورونا. كما تم

العثور على أدلة مصلية في 20 دولة تثبت إصابة الإبل بفيروس كورونا، مع أدلة جزيئية على انتقال الفيروس في 13 دولة. يزيد وجود الأجسام المضادة لفيروس الكورونا مع تقدم العمر في الجمال، بينما يتناقص معدل ذرف الفيروس في مسحات الأنف استناداً لما يحدده الحمض النووي الريبي.

في العديد من الدراسات، كانت الإبل التي تم أخذ عينات منها في أسواق الحيوانات أو مرافق الحجر الصحي إيجابية المصل مقارنة بتلك الموجودة في المزارع وكذلك الإبل المستوردة مقابل الجمال التي تتم تربيتها محلياً. وتشير بعض الدراسات إلى أن معدل اكتشاف الفيروس ورصده مصلياً تعتبر أعلى بشكل نسبي خلال أشهر الشتاء الباردة. إن التعرف على المستودع الحيواني لفيروس الكورونا أمر ضروري لتطوير تدابير التدخل والسيطرة والوقاية من حدوث إصابات بشرية (الفصل الثامن).

ولتحديد ماهية وتأثيرات الأسباب الرئيسية التي يحتمل أن تكون المحركة لنشوء فيروس الكورونا وسريانه في قطر (الفصل الثاني)، استخلصت قائمة بالمحركات المحتملة المتعلقة بكل من الإنسان والحيوان والبيئة من خلال مراجعة المنشورات العلمية وتحليل قواعد البيانات ذات الصلة بجانب استطلاع آراء الخبراء ذوي العلاقة.

وبالنظر إلى تأثير استخراج النفط والغاز واستثماره على مضاعفة الناتج المحلي في الدولة لأكثر من خمس أضعاف، فإن التحسن الذي طرأ تبعاً لذلك أسس لتحول نمط الحياة بشكل تدريجي خلال ثلاثين عاماً من حياة البداوة إلى حياة حضرية يغلب عليها الخمول. كما أن ازدهار أنشطة الترفيه المرتبطة بالإبل كواحدة من الموروثات الثقافية المتجذرة في المجتمع والمدعومة من الدولة أفضى إلى تضاعف أعداد الإبل منذ عام 1990

ومع تفاقم الرعي الجائر وازدياد التصحر بشكل متسارع صدر قرار جرى بموجبه منع الرعي المفتوح منعاً تاماً منذ عام 2005 واستعيض عنه بوضع الإبل في حظائر ضيقة، إلى جوار العديد من الأنواع الأخرى من الحيوانات الأليفة لتعيش جميعها مع العمال الذين يخدمونها في مكان واحد ويضاف إلى ذلك التنقل عبر الحدود بحثاً عن مراعي جديدة. فيعتقد أن تكون هذه التحولات الكبيرة في العادات المتصلة بتربية الإبل قد هيأت الظروف المناسبة للفيروس لينتقل من الجمال للبشر وينتشر في شمال إفريقيا والشرق الأوسط (الفصل التاسع).

تم اقتراح إطار عمل متكامل يجمع بين نهج الصحة الواحدة ومعايير الأمان الحيوي في مزارع الإبل كإستراتيجية عملية لاتقاء الإصابة بفيروس الكورونا والسيطرة عليه واتقاء غيره من الأمراض المشتركة في واجهة الإنسان والحيوان (الفصل 10).

PhD portfolio

Name: Elmoubashar Farag,

Research Group: Department of Viroscience, Erasmus University Medical Center.

PhD Period: 2016- 2019

Promoter: M.P.G. Koopmans

Education Background:

2002, Bachelor of Medicine, Bachelor of Surgery (MBBS), Faculty of Medicine, University of Gezira, Sudan

2007, Master of Public and Tropical Health (MPTH), the University of Medical Sciences & Technology, Khartoum, Sudan

2013, Master of Health Professions Education (JMHP), Maastricht University, The Netherlands

2014, European Master in Disaster Medicine (EMDM), Università del Piemonte Orientale, Vercelli, Italy and Vrije Universiteit Brussel, Belgium

2018, Membership in Travel Medicine (MFTM), Royal College of Surgeon and Physicians of Glasgow, UK

2018, Fellowship in Faculty of Public Health (FFPH), Royal College of Surgeon and Physicians of UK

2019, Master of Clinical Research (MPCR), Dresden International University-DIU, Germany

Other training:

- 2015, Public Health Surveillance, the Johns Hopkins Bloomberg School of Public Health (JHSPH) and the Ministry of Public Health Qatar, Doha, Qatar.
- 2015, Diploma in Epidemiology in Action (EIA), Liverpool School of Tropical Medicine (LSTM), UK
- 2015, Preparedness and Early Response Against Bioterrorism for the Public Health care sector, The national committee for the prohibition of weapon (NCPW), Doha, Qatar
- 2015, Professional in Infection control- American Institute for Health care Quality
- 2015, Planning, Monitoring and Evaluation in Disease Control Programmes, Liverpool School of Tropical Medicine. UK
- 2016, Diploma in Principles and Practices of Clinical Research (PPCR), Harvard T.H. Chan School of Public Health, USA
- 2016, Infectious Diseases Outbreak Investigation workshop, Ministry Of Public health in collaboration with Pasteur Institute (France), Doha, Qatar .
- 2017, Diploma in Travel Medicine, Royal College of Surgeon and Phrygians of Glasgow, UK

- 2018, Diploma in Global Health and Humanitarian Medicine (GHHM), MSF, UK
- 2019, Evidence Based Clinical Practice (EBCP) Certificate, College of Health Sciences, Qatar University, Qatar
- 2018, IATA Infectious Substance Shippers Training (ISST), Doha, Qatar

Professional Experience:

- July 2011 to present: Head of Communicable Diseases Control Programs, Public Health Department, Ministry of Public Health, Qatar
- January 2007 to April 2011: HIV/AIDS Officer at the United Nations Population Fund (UNFPA), Sudan, Khartoum
- January 2006 to December 2006: Head of HIV/AIDS information and communication Unit at the Sudan National AIDS Control Program (SNAP)
- January 2003 to December 2005: Emergency Health Coordinator at KPHF (Kuwaiti Patient Helping Fund) -SUDAN Office

Other activities

1. Health care journalist: Founder and member of the editorial committee of Sahatak Medical journal.
2. January 2006 to present: Professional health care journalist reporting and writing on the health care issues in the different media in Sudan.

List of MERS-CoV Technical meetings, workshops and Conference:

1. 2014, FAO Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in Animal Species Regional Technical Consultation Meeting Muscat, Oman (invited as advisor)
2. 2014, WHO International scientific meetings on MERS-CoV in Riyadh, Saudi Arabia (invited as advisor)
3. 2014, WHO Regional meetings on MERS-CoV, Sharm El Sheikh, Egypt (invited as advisor)
4. 2014, Endemic and Emerging Viral Diseases of Priority in the Middle East and North Africa (MENA): NIH-QNRF Scientific Workshop; Doha, Qatar (Poster presentation)
5. 2014, International Meeting on Emerging Diseases and Surveillance-IMED. Vienna, Austria (Poster presentation)
6. 2015, FAO and the State of Qatar in collaboration with OIE and WHO “ Regional workshop on MERS-CoV and ‘One Health’, Doha, Qatar (member of the organizing committee and presenter)
7. 2015, The 2nd International One Health Conference of the One Health Central and East Africa (OHCEA) “Strategic Approach to Global Health Security Through One Health Innovations: Vision 2035” Kampala, Uganda (invited as speaker)
8. 2015, 3rd International One Health Congress , Amsterdam, The Netherlands (invited as presenter)

9. 2015, The Regional Conference of Camel Management and Production under Open range System (RCCMPR), Khartoum, Sudan (invited as presenter)
10. 2015, The International Symposium on MERS from in Seoul, Republic of Korea (invited as presenter)
11. 2015, The International Conference on Emerging Infectious Diseases 2015 (ICEID 2015) at the Hyatt Regency Hotel in Atlanta, Georgia, USA (invited as presenter)
12. 2016, 4th Global Influenza Sero-epidemiology Expert Meeting, Institute Pasteur, Paris, France (invited as speaker)
13. 2016, The Technical Meeting on Understanding MERS-CoV at the animal-human interface, Rome, Italy (Invited as advisor).
14. 2016, Infectious Diseases Outbreak Investigation, Ministry Of Public health in collaboration with Pasteur Institute (France), Doha, Qatar (Member of the organizing committee and presenter)
15. 2016, Workshop on “Emerging Pathogens at the Human-Animal-Environment Interface”. Qatar University Biomedical Research Centre (QU-BRC) and Qatar National Research Fund (QNRF), Doha, Qatar (Member of the organizing committee and presenter)
16. 2016, First International Conference in Emergency Medicine and Public Health–Qatar (ICEP-Q 2016), Doha, Qatar (Member of the organizing committee and presenter)
17. 2017, WHO Meeting on the Environmental Contamination Studies of MERS-CoV in Health Care Settings co-hosted by WHO and The University of Hong Kong (Invited as advisor and presenter)
18. 2017, The Global Outbreak Alert and Response Network-GOARN technical workshop on MERS-CoV Training Curriculum Co-hosted by WHO and The University of Hong Kong (Invited as advisor and presenter)
19. 2017, FAO-OIE-WHO Global Technical Meeting on MERS-CoV, WHO Headquarters Geneva, Switzerland (Invited as advisor and presenter)
20. 2017, The 4th International Congress on Pathogens at the Human-Animal Interface (ICOPHAI), Environmental Challenges and Impact on Global Health, Doha, Qatar. (Organizing committee member and presenter)
21. 2018, WHO-IVI Joint Symposium for MERS-CoV Vaccine Development, Seoul, South Korea (Invited as advisor).
22. 2017, WHO Intercountry consultation on Mass Gathering Preparedness and Management, Jakarta, Indonesia (Invited as advisor)
23. 2017, WHO and Global Center for Mass Gathering Medicine (GCMGM) WHO Technical Consultation on Health Risk Assessment Tools for Mass Gatherings Events, Jeddah, Saudi Arabia (Invited as advisor)
24. 2018, International Meeting on Emerging Diseases and Surveillance IMED. Vienna, Austria (Participant)

25. 2018, WHO Workshop “Efficacy trials of MERS-CoV Therapeutics and Vaccines: endpoints, trial design, site selection”, CCV, Geneva, Switzerland (Invited as advisor).
26. 2018, Molecular training for diagnostic MERS CoV detection at the Blue Nile Institute in Wad Madani-Sudan (organizing committee member and presenter)
27. 2018, Trends of Intestinal Parasitic Infections in Qatar Seminar, Ministry of public health-MOPH Auditorium, Doha, Qatar (organizing committee member and presenter)
28. 2018, The Regional Case-studies Development Workshop/ GHD and The Eastern Mediterranean Public Health Network (EMPHNET)-EMPHNET, Amman, Jordan (Invited as advisor and presenter)
29. 2018, Data sharing practices in public health emergencies - learning lessons from past outbreaks workshop, Wellcome Trust, London, UK (invited participant)
30. 2018, WHO Experts meeting on “one-health framework for action” for emerging zoonotic infections, Amman, Jordan (Invited as as advisor and presenter)
31. 2018, WHO Inter-country Meeting on Strategic Framework for Prevention and Control of Emerging and Epidemic Prone Diseases in the Eastern Mediterranean Region, Amman (Invited as as advisor and presenter)
32. 2018-2019, a Tripartite (FAO, OIE and WHO) workgroup meetings to update and expand the 2008 Tripartite Zoonoses Guide (TZG) and to develop Surveillance and Information Sharing tools for the new Tripartite Zoonoses Guide. (Invited as advisor).

Curriculum vitae

My name is Elmoubasher Farag. Born in Sudan, in 1975. I started my professional journey since I was yet a medical student in Al Gazeera University. I designed the first structured community outreach program in 1997, an initiative that got the attention of the faculty who picked me afterwards to join an unprecedented committee tasked with reviewing the curriculum a year later. Fully inspired by the impact of such valued contributions to the public health I dared to design two successive community-based surveys. While the first screened the farmers for Bilharzia, the other studied Vitamin A deficiency among school children. I am much proud that the results of these surveys were used by Ministry of Health to implement comprehensive treatment and prevention projects. The success I found in these surveys encouraged me to lead two more surveys for oral health and maternal health in rural community. Just before my graduation from the school of medicine in 2002, I crowned my undergraduate heritage with establishing a network of community health committees across the rural areas of Al Gezira State. I ended my time in the school of medicine, with a firm belief that the community medicine will be the only discipline to accommodate my aspirations and personal dreams.



While fulfilling my internship as a houseman officer, I did not abandon my inclination towards public health and research. I was keen to observe how the lack of effective preventive strategies would unpleasantly display in medical problems the way we ultimately see in the referral hospitals. I was preoccupied by variety of thoughts as to what role I can assume to make a substantial difference. I thought I would help my medical colleagues get more involved in the research where they can recognize the disease determinants, I founded “the Nile Center for Medical Ethics” in Sudan in 2003-2005 to serve as a center for research and capacity building in the field. I even created “Sahatak”, a medical magazine addressing the local health problems of Sudanese people, to help highlighting the health problems from preventive perspective to both the medical community and the public. My magazine has just completed its 14th birthday, with a decent readership. I am most content with such an achievement that would only need me to initiate, then survive regardless my involvement. Another dream that comes true.

I carried my public health passion over when I joined Sudan National AIDS Control Program (SNAP) at the Federal Ministry of Health and played a leading role in reviewing of the HIV/AIDS control guidelines in emergencies like Darfur, where the funds I raised managed to get two grants from the Global Fund with total of seven million US\$. I further designed and led the first national integrated bio-behavioral survey for HIV/AIDS Most-at-risk Population in Sudan in 2010 to inform the control and prevention policy and strategies targeting the most at risk groups.

In 2010, I had two cardinal achievements that I hoped would serve generations to come. Still inspired by my previous work in designing a community-oriented curriculum, I developed the ‘Community Health Institute’ for the Faculty of medicine, Omdurman Islamic University in addition to developing the HIV AIDS professional higher Diploma, for the University of Medical Science and Technology -Sudan.

A remarkable leap on my career occurred when I joined the Ministry of Public Health in Qatar. I learnt that I would be supervising the disease control division, where I would be required to draft Qatar National Health Strategy for communicable diseases 2011-2016 and also to prepare the first framework for prevention and control of communicable diseases. I discerned that as workers make more than 70% of the population in Qatar, addressing this group with a tangible strategic initiative would make a significant difference to the profile of communicable diseases in the country. I innovated ‘Wiqaya’, an initiative to promote hygienic and health-seeking practices among the male workers in Qatar. Wiqaya success was not only evident in the remarkable interaction it generated among the target workers, but also in the engagement of the local partners (like the Qatari Red Crescent) who found it appealing to make it rather a sustained program.

As the lead in investigating the Middle East Respiratory Syndrome Corona Virus-MERS-COV suspected cases, I helped confirm the camels’ role in the transmission of the infection, marking one of the major breakthroughs of the disease. Later, I planned and conducted a national MERS CoV sero survey among the group-at-risk.

Maybe it has been obvious that I am biased to bringing research and capacity building to the work in which I get involved. While I admit such kind of inclination, I strongly believe that the back bone to the public health, as well as to the other healthcare lines of specialty, is the competent human resources who are guided by science. This might explain my keenness to develop reliable healthcare professionals to support quality service delivery. I managed somehow to translate my aspirations into actions.

I succeeded in convincing the MoPH decision makers to adopt a wide range of capacity building short courses in collaboration with Liverpool School of Tropical Medicine, the WHO, The CDC Atlanta, Erasmus Mc Institute, Pasteur Institute, Harvard School of Public Health, and the USA National Institute of Health. Since 2011 up to date, I have been affiliated as instructor for several public health and medicine training programs. I also have teaching contribution to Qatar University, Weil Cornell School of Medicine-Qatar, and College of North Atlantic. I was nominated to be 'Qatar site monitor' for Harvard Training Program in clinical research as well as being the site director of the online training program in clinical research of the NIH.

I am proud that I succeeded since 2011 up to date to get 4 research grants with total of three Million US\$ from Qatar Foundation to address public health priorities in Qatar. Similarly, I lead 10 research projects all were funded by the Ministry of Public Health in Qatar between 2011 till 2019. The outcome of such research projects are around 40 published paper and 25 conference participation.

Despite the burden of professional responsibilities, I managed to pursuit three Masters Degrees, and four diplomas in addition to membership in Travel Medicine, Royal College of Surgeon and Phrygians of Glasgow, besides fellowship of the Faculty of Public Health, Royal College of Surgeon and Physicians of UK.

List of publication

1. **Farag EA**, Haagmans BL, Al-Romaihi HE, et al. Failure to detect MERS-CoV RNA in urine of naturally infected dromedary camels. *Zoonoses Public Health*. 2019; 00:1–2. <https://doi.org/10.1111/zph.12583>
2. Widagdo Widagdo, Nisreen Mamdouh Ahmed Okba, M. Richard, D. de Meulder, T. M. Bestebroer, P. Lexmond, **Elmoubasher Farag**, M. Al-Hajri, Koert Stittelaar, Leon de Waal, Geert van Amerongen, Judith M A van den Brand, Bart L Haagmans, Sander Herfst: Lack of Middle East Respiratory Syndrome Coronavirus Transmission in Rabbits. *Viruses* 04/2019; 11(4):381., DOI:10.3390/v11040381
3. **Farag, E.**, M. Nour, M. M. Islam, A. Mustafa, M. Khalid, R. S. Sikkema, F. Alhajri, A. Bu-Sayaa, M. Haroun, M. D. Van Kerkhove, A. Elkholy, S. M. R. Malik, C. Reusken, M. Koopmans and M. M. AlHajri (2019). “Qatar experience on One Health approach for middle-east respiratory syndrome coronavirus, 2012–2017: A viewpoint.” *One Health* 7: 100090. doi:10.1016/j.onehlt.2019.100090
4. **Elmoubasher Farag**, Mohamed Nour, Ahmed El Idrissi, Jaouad Berrada, Aya Moustafa, Minahil Mehmood, Mahmoud H Mahmoud, Ahmed M El-Sayed, Farhoud Alhajri, Mohammed Al-Hajri, Osama Ahmed Hassan, Hamad Al-Romaihi, Mohamed Al-Thani, Salih A Al-Marri, Marion P G Koopmans, Mohamed Haroun Ismail: Survey on Implementation of One Health Approach for MERS-CoV Preparedness and Control in Gulf Cooperation Council and Middle East Countries. *Emerging Infectious Diseases* 03/2019; 25(3), DOI:10.3201/eid2503.171702
5. Reina Sikkema, **Elmoubasher Farag**, Md Mazharul Islam, Muzzamil Atta, Chantal B. E. M. Reusken, Mohd M. Alhajri, Marion Koopmans: Epidemiology and Infection Global status of Middle East respiratory syndrome coronavirus in dromedary camels: a systematic review. *Epidemiology and Infection* 02/2019;, DOI:10.1017/S095026881800345X
6. Reham A. Al Kahlout, Gheyath K. Nasrallah, **Elmoubasher Farag**, Lingshu Wang, Erik Lattwein, Marcel A. Müller, Mohamed E. El Zowalaty, Hamad E. Al Romaihi, Barney S. Graham, Asmaa A. Al Thani, Hadi M. Yassine: Comparative Serological Study for the Prevalence of Anti-MERS Coronavirus Antibodies in High- and Low-Risk Groups in Qatar. *Journal of Immunology Research* 02/2019; 2019(6):1-8., DOI:10.1155/2019/1386740
7. **Elmoubasher Farag**, Reina S Sikkema, Tinka Vinks, Md Mazharul Islam, Mohamed Nour, Hamad Al-Romaihi, Mohammed Al Thani, Muzzamil Atta, Farhoud H Alhajri, Salih Al-Marri, Mohd Alhajri, Chantal Reusken, Marion Koopmans: Drivers of MERS-CoV Emergence in Qatar. *Viruses* 12/2018; 11(22), DOI:10.3390/v11010022

8. Sarah Cabalion, **Elmoubasher Farag**, Omer Abdelahdi, Hamad Al-Romaihi, Frédéric Keck: Middle East respiratory syndrome coronavirus and human-camel relationships in Qatar. 06/2018; 5(3):177. DOI:10.17157/mat.5.3.377
9. Mohamed Nour, Mohd Alhajri, **Elmoubasher Farag**, Hamad E. Al-Romaihi, Mohamed Al-Thani, Salih Al-Marri, Elena Savoia: How Do the First Days Count? A Case Study of Qatar Experience in Emergency Risk Communication during the MERS-CoV Outbreak. International Journal of Environmental Research and Public Health 12/2017; 14(12):1597., DOI:10.3390/ijerph14121597
10. Reina S Sikkema, **Elmoubasher Farag**, Sayed Himatt, Adel K Ibrahim, Hamad Al-Romaihi, Salih A Al-Marri, Mohamed Al-Thani, Ahmed M.H. El-Sayed, Mohammed Al-Hajri, Bart L Haagmans, Marion P.G. Koopmans, Chantal B.E.M. Reusken: Identification of risk factors for primary MERS-CoV infection in camel workers in Qatar, 2013 - 2014; a case-control study. The Journal of Infectious Diseases 04/2017; 215(11)., DOI:10.1093/infdis/jix174.
11. K.A. Mohran, **E. Farag**, C.B.E.M. Reusken, V.S. Raj, M.M. Lamers, S.D. Pas, J. Voermans, S.L. Smits, M.M. AlHajri, F. Alhajri, H.E. Al-Romaihi, H. Ghobashy, M.M. El-Maghraby, S.H.S. Al Dhahiry, N. Al-Mawlawi, A.M. El-Sayed, M. Al-Thani, S.A. Al-Marri, B.L. Haagmans, M.P.G. Koopmans: The sample of choice for detecting Middle East respiratory syndrome coronavirus in asymptomatic dromedary camels using real-time reverse-transcription polymerase chain reaction. Revue scientifique et technique (International Office of Epizootics) 12/2016; 35(3):905-911., DOI:10.20506/rst.35.3.2578
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13. **E. Farag**, M. nour, O. Marufu, R. Sikkema, H. Al-Romaihi, M. Al Thani, S. Al-Marri, A. El-Sayed, C.B.E.M. Reusken, M. Al-Hajri, M.P.G. Koopmans: The hidden epidemic: MERS-CoV-related stigma observations from the field, Qatar 2012-2015. International Journal of Infectious Diseases 04/2016; 45(1):332., DOI:10.1016/j.ijid.2016.02.719
14. Chantal B.E.M. Reusken, Chrispijn Schilp, V. Stalin Raj, Erwin De Bruin, Robert H.G. Kohl, **Elmoubasher Farag**, Bart L. Haagmans, Hamad Al-Romaihi, Francois Le Grange, Berend-Jan Bosch, Marion P.G. Koopmans: MERS-CoV infection of alpaca in a region where MERS-CoV is endemic. Emerging Infectious Diseases 04/2016; 22(6)., DOI:10.3201/eid2206.152113
15. Chantal B. E. M. Reusken, **Elmoubasher Farag**, Bart L. Haagmans, Khaled A. Mohran, Gert-Jan Godeke, V. Stalin Raj, Farhoud Alhajri, Salih A. Al-Marri, Hamad E. Al-Romaihi, Mohamed Al-Thani, Berend-Jan Bosch, Annemiek A. Van Der Eijk, Ahmed M. El-Sayed, Adel K. Ibrahim, N. Al-Molawi, Marcel A. Muller, Syed K. Pasha, Christian Drosten, Mohd M. Alhajri, Marion P. G. Koopmans: Occupational Exposure to Dromedaries

- and Risk for MERS-CoV Infection, Qatar, 2013-2014. *Emerging Infectious Diseases* 08/2015; 21(08):0481., DOI:10.3201/eid2108.150481
16. **Elmoubasher Farag**, Chantal B E M Reusken, Bart L Haagmans, Khaled A Mohran, V. Stalin Raj, Suzan D Pas, Jolanda Voermans, Saskia L Smits, Gert-Jan Godeke, Mohd M Al-Hajri, Abcm, Farhoud H Alhajri, Hamad E Al-Romaihi, Hazem Ghobashy, Mamdouh M El-Maghraby, Ahmed M El-Sayed, Mohamed H J Al Thani, Salih Al-Marri, Marion P G Koopmans: High proportion of MERS-CoV shedding dromedaries at slaughterhouse with a potential epidemiological link to human cases, Qatar 2014. *07/2015*; 5:28305., DOI:10.3402/iee.v5.28305
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 18. Eun Kim, Kaori Okada, Tom Kenniston, V Stalin Raj, Mohd M AlHajri, **Elmoubasher Farag**, Farhoud AlHajri, Albert D.M.E. Osterhaus, Bart L Haagmans, Andrea Gambotto: Immunogenicity of an adenoviral-based Middle East Respiratory Syndrome coronavirus vaccine in BALB/c mice. *Vaccine* 09/2014;, DOI:10.1016/j.vaccine.2014.08.058
 19. Stalin Raj V, **Farag E**, Reusken CBEM, Mart M. Lamers, Suzan D Pas, Jolanda Voermans, Saskia L Smits, Albert D M E Osterhaus, Naema Al-Mawlawi, Al-Romaihi HE, AlHajri MM, El-Sayed AM, Khaled A Mohran, Hazem Ghobashy, Farhoud Alhajri, Mohamed Al-Thani, Al-Marri SA, El-Maghraby MM, Marion P G Koopmans, Bart L Haagmans: Isolation of MERS Coronavirus from a Dromedary Camel, Qatar, 2014. *Emerging Infectious Diseases* 08/2014; 20(8)., DOI:10.3201/eid2008.140663
 20. C B Reusken, **Farag E**, Jonges M, G J Godeke, El-Sayed AM, S D Pas, V S Raj, Mohran KA, Moussa HA, Ghobashy H, Alhajri F, A K Ibrahim, B J Bosch, S K Pasha, Al-Romaihi HE, Al-Thani M, Al-Marri SA, AlHajri MM, B L Haagmans, M P Koopmans: Middle East respiratory syndrome coronavirus (MERS-CoV) RNA and neutralising antibodies in milk collected according to local customs from dromedary camels, Qatar, April 2014. *Eurosurveillance: bulletin europeen sur les maladies transmissibles = European communicable disease bulletin* 06/2014; 19(23):20829., DOI:10.2807/1560-7917.ES2014.19.23.20829
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“Praise be to Allah who is done with His grace”

Alhamdulillah, I praise and thank Allah for His greatness and for giving me the Strength and courage to complete this thesis.

It was Eid Al Adha (or feast of sacrifice), the biggest annual Eid (festival) for Muslims. On the second day and while we were enjoying the breakfast with the family, Dr. Hamad Al Romaihi, surveillance section head at that time, unexpectedly called me to investigate a case suspected with MERS. I had to assemble the ‘Rapid Response Team’ as well as another team from the Animal Health Department to initiate a joint outbreak investigation. We headed to Al Sheehaniya area to undertake a mission prevailed with a great deal of uncertainty.

After several hours in an extremely hot and humid weather we still had no clue what to do and what samples to take. The joint investigation team got bored and even some of them decided to abruptly flee the investigation scene as the strenuous efforts seemed pointless. Eight hours spent until we arrived at a decision to collect samples from everything. Our team was unfamiliar with such kind of extraordinary act. Even some of my colleagues refused to take samples from camels. Fortunately, the required samples along with the epidemiological data were collected.

Human samples (urine, blood, respiratory swabs), soil, grass, water, animal samples (blood, feces, respiratory samples) were all collected then shipped to Hamad Medical Corporation Laboratories. At first, Dr. Saeed Al Dhahiri was hesitant to store animal and environmental specimens into his lab. Thanks to his investigative mind, he finally approved to keep them until they are shipped to an external reference lab. Days later, the WHO and FAO conducted their joint visit. Where Dr. Peter Benembark from FAO made the first contact with Professor Marion Koopmans, the head of the viruscience department, Erasmus MC in the Netherlands.

Thereafter, Bart Haagmans, supported by his team from Erasmus MC, has thankfully helped detect MERS-CoV, providing the very first proof that camels are to be linked to MERS-CoV infection. Later, Stalin managed to isolate MERS-CoV from samples taken from Qatar camels. Then, Chantal. Reusken provide the world with more evidences about mers. cov at Human animal Interface.

It was all about patience and determination to bring answers. Big thanks for the joint rapid response team, without their efforts and patience nobody knows how many persons could have been affected before we determine MERS-CoV source. It all depended on the one-health (the key that enabled the epidemiological breakthrough where principles of epidemiology were applied).

These breakthroughs and discoveries paved the way to seriously pursuing a high degree while finding answers to MERS-CoV epidemic. Risk assessment and samples collection continued until I was destined to take part in the consultative meeting about MERS-CoV meeting, KSA 2014. It was the first time to meet up face to face with Professor Marion Koopmans. She provided the world with further evidence about MERS-CoV from samples collected from camels and vulnerable groups in Qatar. I felt more confident and enthusiastic to go for it. Unknowing how would Prof Marion Koopmans would respond to the idea, I voiced my proposal to compile my scientific perspectives and papers as a PhD. Her welcoming of the notion was incomparable. She then was my mentor who supervised me over the course of 4 years.

As the PhD is a remarkable life achievement for the sake of which so many sacrifices has been made, I believe it avails a unique chance to reflect on one's experiences and the people happened to provide help. I really like this chapter where it permits to derail the paradox of firm scientific language to emotional terms in the hope to express my gratitude to those without whom this work could have never see the light.

Couple of characteristics made Professor Koopmans supervision to my research an exceptional experience; her intensive knowledge about the research subject besides her deep familiarity with the emerging infectious diseases and MERS-CoV and Qatar cultural context. It is difficult to imagine anybody, but her, to competently guide me through the puzzling turns of my project. A million of thanks goes to her a (Veterinary Doctor working at a human hospital).

Another unique person that I was lucky to work with is Chantal. I learnt that she loves the sea but the desert always captures her. Whenever I meet with her, I admire that she seems never content from exploration. No wonder why I benefitted from her critical contemplation of the epidemiological theories and the virus behavior. Chantal was the one sent to meet us in Qatar, critical thinking and interrogative mentality. Her leadership, dedication, and commitment made her an extraordinary qualified co-investigator in the Qatar-Netherlands MERS-CoV project.

Reina Sikkema, who is smart, well organized and an active member of the MERS-CoV study group, was so kind in offering her advice that helped achieve well in my research. I believe she will be an 'emerging' leader in the field of emerging infectious diseases and the one-health.

I have to acknowledge Ms. 'Loubna Bouzyd', secretary of the viroscience department at EMC for her continuous support and guidance. I am also grateful to Simone Slabbekoorn and Maria Silva for their kind care and supportive treatment. In fact I need to thank the entire viroscience department for considering me as one of their family.

There are so many persons to thank in Qatar. My former manager, Dr. Mohammed Al Hajri, the National lead for Mers.cov preparedness and response in Qatar, deserve a special thanks for his unparalleled welcoming to ideas no matter how bold or extraordinary. With his famous life slogan "sky is the limit," he steadily kept devoting the credit of several

breakthroughs that were achieved under his leadership to me. Dr. Hamad Al Romaihi, my current manager at the HP & CDC department, was rather a friend than a boss. I am very grateful to his support without which neither resources nor staff could have been made available.

I am personally indebted to both Dr. Salih Al Marri, the Undersecretary of Health and ShK. Dr. Mohamed Al Thani, the Director General of the Public Health Department, Ministry of Public Health. As their leadership and guidance in fact laid the foundation to literally all the achievement I am proud of in Qatar. Such achievement could have never happen unless they adopted a proactive policy of data-driven, none of the papers that made up my PhD thesis could have seen the light.

Words will fall short from thanking my colleagues staff of the HP & CDC department. In particular I would like to mention Dr. Ahmed El Sayed, Mr. Fareed Shehata, Mr. Khalid Yousif, and Mr. Thomas Kutty in addition to the ARDS and the active surveillance team who all burdened the strenuous field work in so harsh conditions to interviewing suspected cases, collecting samples and being courageous to continue exposing themselves to the risk of the virus even when uncertainty about his transmission was prevailing.

I am using this occasion to express my appreciation to my colleagues who supported me during the course of this PhD project. I am grateful for their ambitious guidance, invaluable constructive criticism and friendly assistance during the project work. I express my wholehearted thanks for my colleagues Dr. Mohamed Nour, Dr. Mazharul Islam, Dr. Dev Bansal and Prof Mohammed ismail for their support. I am sincerely thankful to them for sharing their enlightening visions on an amount of issues related to this research project.

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For a good reason I believe my devotion stemmed from my parents. (Dad Osman Abdu and Mum Amna Hassan) both discerned my passion to explore and learn. No words can describe my gratitude to their sacrifices to pave the roads ahead of my future. I am nothing without them. With their unconditional persistent support and motivation, my sisters; Maha, Manal and Zahra, pushed me to succeed. The appreciation of my only brother, Muaz, was in itself a kind of untold motive to continue my journey.

Similarly, my uncles 'Yousif' and 'Omer', who, in an uncountable times, helped me take informed decisions pertaining my schooling and study disciplines. Looking at how far I managed to reach now, I can tell their advices proved right, every time. The circle of influence was arguably, however, bigger than just my close family. My neighbors and acquaintances in 'New Halfa' in Eastern Sudan, the small town where I grew up. We were a

group of distinguished students who usually score high grades. I cannot forget the peer-to-peer competition that featured our relationship as young pupils. 'Mohammed Asaad' and 'Aymen Hilal' still leading successful life since that beautiful time.

Like our fathers, our teachers at the primary, intermediate, then secondary school Mr. Abdelhameed, Mr. Mohamed, Mr. Ali, Mr. Sulieman, Mr Abdel Mageed were able to see through our insides to help us get over our internal struggles. They have had a lasting effect to my soul and morals. The taste of success was in itself rewarding, but the encouragement of 'New Halfa' community was undeniable.

Another unique teacher, yet a friend, was Sheikh 'Mohamed Sayed Haj. A man that you can be sure was 'one of a kind.' With his robust knowledge in Quraan and Sunna sciences, Sheikh Mohamed was the light who opened my eyes to take medicine as a career. He strongly believed that I will excel in this field. He advised that I work hard to help those in pain to please the Almighty God. I was inspired by his visions and words. Similarly, Sheikh 'Yagoub' and Sheikh 'Al Tahir' who equipped me with golden advices about having a good faith in 'Allah' and his blessings as they recognized my eagerness to excel for the sake of the public.

Al Gazeera University was another story in my life. I am privileged with professors who welcomed my eagerness to sometimes work with them in 'extracurricular projects and programs.' The system in Al Gazeera medical school from where I was graduated allowed for, rather encouraged, such kind of activities that can discover and polish the students' capabilities. All of my professors deserve my thanks but I would like to mention Prof 'Sameera Hamid', Prof 'Omer Aziz'. Yet, my senior colleagues, namely 'Mutaz Urabi' and 'Azza Zulfo', had a significant role in offering their advices which helped me achieve a steady performance in my medical school through graduation. Not to forget my colleagues in batch 18. This batch was characterized for it high, yet noble, competition. Such competition has positively influenced my career.

Life has driven me from experience to another. But joining the Patients' Helping Fund-None Governmental Organization was a distinctive one. The spirit of 'giving' could easily be appreciated from the humanitarian projects supervised by Dr. Mohamed Al Hassan Abdelrahman, the PHF Director. Famous with his second name, Dr. Hassan, was one of few who believed in my innovation competences as I was engaged in planning for humanitarian projects. He even extended his support beyond the organization projects to support my participation in the international scientific conferences. I need also to underscore the sessions of free brain storming with Dr. Kamal Yagroub, the Deputy Director for PHF. Through his support to 'thinking outside the box,' Dr. Kamal, along with my friends Mr. Mohamed Omer Husein, Mr. Mutasim Alameen, and my colleagues, motivated me with a good cause to audaciously pioneer in inventing unparalleled programs. They have thankfully offered their offices, internet, and even lab tops, when these resources were so precious. 'El Nile Center for Medical Ethics' and 'Sahatak Health Magazine' were examples of several projects generated out of such firm support and encouragement.

A distinguished expert in public health that would never have an equivalent to is Dr. 'Elwathiq Al Hamadabi.' When I was not sure about the medical career, his insightful consultation has remarkably helped me take one of the most critical decisions in my life and career. I am so appreciative to him and to Dr. Ginawi, who both persuaded me to invest my efforts in the public health field which is rich with so many virgin areas to welcome my enthusiasm to excel and innovate. Likewise, Ms. Wifaq Salah, a former supervisor at the UNFPA office, Sudan who believed that my passion to public health and research will help me achieve a unique mission.

Of course, my little family deserve sincere regards. My wife, 'Nafisa Hummaida' , earnt the hard way how to live a life of an epidemiologist who is passionate about research. As my passion takes me from home, she remained supportive, shouldering the burden of looking after our kids and managing the house. Very few women can really take that part, silently. My kids, Rawah, Anas, Osman, Ahmed and Abdel Rahman, all paid their share in my successes from their times they deserved be spent with them. For so many times they sacrificed their entertainment time and kept quiet at home not to disturb my work in the research.

List of co-authors

Abdullatif Al-Khal

Hamad Medical Corporation, Doha, Qatar

Abdulla Bu-Sayaa

Department of Animal Resources, Ministry of Municipality and Environment, Doha-Qatar

Adel K. Ibrahim

Leawaina Camel Hospital, Dukhan, Qatar

Ahmed El Idrissi

Food and Agriculture Organization of the United Nations, Rome, Italy

Ahmed M. El-Sayed

Ministry of Public Health, Doha-Qatar

Albert D.M.E. Osterhaus

Erasmus Medical Center, Rotterdam, The Netherlands

Amgad Elkholy

Infectious Hazard Management, Department of Health Emergency, World Health Organization, Eastern, Mediterranean Regional Office, Cairo, Egypt

Annemiek A. van der Eijk

Erasmus Medical Center, Rotterdam, The Netherlands

Aya Mustafaa

Ministry of Public Health, Doha-Qatar

Bart L. Haagmans

Erasmus Medical Center, Rotterdam, The Netherlands

Berad Jan Bosch

Utrecht University, Utrecht, the Netherlands

Chantal B. E. M. Reusken

Erasmus Medical Center, Rotterdam, The Netherlands

Christian Drost

University of Bonn Medical Center, Bonn, Germany

Devendra Bansal

Ministry of Public Health, Doha-Qatar

Farhoud H. Alhajri

Department of Animal Resources, Ministry of Municipality and Environment, Doha-Qatar

Gert-Jan Godeke

Center for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, the Netherlands

H. A. Moussa

Leawaina Camel Hospital, Dukhan, Qatar

Hamad Eid Al-Romaihi

Ministry of Public Health, Doha-Qatar

Hazem Ghobashy

Department of Animal Resources, Ministry of Municipality and Environment, Doha-Qatar

Jamila Al Ajmi

Hamad Medical Corporation, Doha, Qatar.

Jaouad Berrada

institut Agronomique et Vétérinaire Hassan, Rabat, Morocco

Jolanda Voermans

Erasmus Medical Center, Rotterdam, The Netherlands

Khaled A. Mohran

Department of Animal Resources, Ministry of Municipality and Environment, Doha-Qatar

Khalid Elawad

Primary Health Care Corporaton, Doha, Qatar.

M. Jonges

Center for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, The Netherlands

Mahmoud H. Mahmoud

Department of Animal Resources, Ministry of Municipality and Environment, Doha-Qatar

Mamdouh M. El-Maghraby

Department of Animal Resources, Ministry of Municipality and Environment, Doha-Qatar

Marcel A. Müller

University of Bonn Medical Center, Bonn, Germany

Maria D. Van Kerkhove

Global Infectious Hazards Management, Health Emergencies Program, World Health Organization, Geneva, Switzerland

Marion P. G. Koopmans

Erasmus Medical Center, Rotterdam, The Netherlands

Mart M. Lamers

Erasmus Medical Center, Rotterdam, The Netherlands

Md. Mazharul Islam

Department of Animal Resources, Ministry of Municipality and Environment, Doha-Qatar

Minahil Khalid

Ministry of Public Health, Doha-Qatar

Mohamed Hamad J. Al-Thani

Ministry of Public Health, Doha-Qatar

Mohamed Haroun

Ministry of Public Health, Doha-Qatar

Mohamed Nour

Ministry of Public Health, Doha-Qatar

Mohammed M. Al-Hajri

Ministry of Public Health, Doha-Qatar

Muna Muslemani

Hamad Medical Corporation, Doha, Qatar.

Muzammil Atta

Department of Animal Resources, Ministry of Municipality and Environment, Doha-Qatar

Naema Al-Mowlawi

Hamad Medical Corporation, Doha, Qatar

Osama Ahmed Hassan

Center for Global Health of Oslo University, Oslo, Norway

Peter Coyle

Hamad Medical Corporation, Doha, Qatar.

Reina S. Sikkema

Erasmus Medical Center, Rotterdam, The Netherlands

Salih Ali Al-Marri

Ministry of Public Health, Doha-Qatar

Saskia L. Smits

Erasmus Medical Center, Rotterdam, The Netherlands

Sayed Himatt

Ministry of Public Health, Doha-Qatar

Sk. Mamunur R. Malik

Infectious Hazard Management, Department of Health Emergency, World Health Organization, Eastern, Mediterranean Regional Office, Cairo, Egypt

Suzan D. Pas

Erasmus Medical Center, Rotterdam, The Netherlands

Syed K. Pasha

Leawaina Camel Hospital, Dukhan, Qatar

Tinka Vinks

Institute of Risk Assessment Sciences, Faculty of Veterinary Medicine, Yalelaan 2, 3584 CM Utrecht, The Netherlands

Victor Stalin Raj

Erasmus Medical Center, Rotterdam, The Netherlands

