Virus Genes
DOI 10.1007/s11262-016-1367-1

Epidemiological investigation of Middle East respiratory syndrome coronavirus in dromedary camel farms linked with human infection in Abu Dhabi Emirate, United Arab Emirates

Salama Al Muhairi[1] · Farida Al Hosani[2] · Yassir M. Eltahir[3] · Mariam Al Mulla[2] · Mohammed F. Yusof[1] · Wissam S. Serhan[1] · Farouq M. Hashem[1] · Elsaeid A. Elsayed[4] · Bahaaeldin A. Marzoug[4] · Assem S. Abdelazim[4]

Received: 23 March 2016 / Accepted: 21 June 2016

Abstract The objective of this research was to investigate the prevalence of Middle East respiratory syndrome coronavirus (MERS-CoV) infection primarily in dromedary camel farms and the relationship of those infections with infections in humans in the Emirate of Abu Dhabi. Nasal swabs from 1113 dromedary camels (39 farms) and 34 sheep (1 farm) and sputum samples from 2 MERS-CoV-infected camel farm owners and 1 MERS-CoV-infected sheep farm owner were collected. Samples from camels and humans underwent real-time reverse-transcription quantitative PCR screening to detect MERS-CoV. In addition, sequencing and phylogenetic analysis of partially characterized MERS-CoV genome fragments obtained from camels were performed. Among the 40 farms, 6 camel farms were positive for MERS-CoV; the virus was not detected in the single sheep farm. The maximum duration of viral shedding from infected camels was 2 weeks after the first positive test result as detected in nasal swabs and in rectal swabs obtained from infected calves. Three partial camel sequences characterized in this study (open reading frames 1a and 1ab, Spike1, Spike2, and ORF4b) together with the corresponding regions of previously reported MERS-CoV sequence obtained from one farm owner were clustering together within the larger MERS-CoV sequences cluster containing human and camel isolates reported for the Arabian Peninsula. Data provided further evidence of the zoonotic potential of MERS-CoV infection and strongly suggested that camels may have a role in the transmission of the virus to humans.

Keywords Middle East respiratory syndrome coronavirus · Dromedary camel · Zoonosis

Edited by Zhen F. Fu.

✉ Salama Al Muhairi
salama.almuhairi@adfca.ae

[1] Veterinary Laboratories Division, Animal Wealth Sector, Abu Dhabi Food Control Authority, Abu Dhabi, United Arab Emirates

[2] Department of Communicable Diseases, Public Health and Research, Abu Dhabi Health Authority, Abu Dhabi, United Arab Emirates

[3] Epidemiology Section, Animal Wealth Sector, Abu Dhabi Food Control Authority, Abu Dhabi, United Arab Emirates

[4] Veterinary services Section, Animal Wealth Sector, Abu Dhabi Food Control Authority, Abu Dhabi, United Arab Emirates

Introduction

Middle East respiratory syndrome coronavirus is a human coronavirus identified in 2012 [1]. Data from recent MERS-CoV outbreak investigations suggest that camels could be a source of human infections [2]. MERS-CoV sequences obtained from humans and camels in Oman, Qatar, and the Kingdom of Saudi Arabia revealed a close link between the virus detected in camels and that detected in people in the same geographic area [3–5]. However, the exact route of transmission from camels to humans remains unclear. MERS-CoV possible routes of transmission in humans and camels include camel-to-human, human-to-human, camel-to-camel, and human-to-camel. [6]. It is worth noting that the presence of the evolutionarily conserved DPP4 protein as a viral receptor suggests that not only dromedary camels, but also other animal species may be potential targets of this virus [7].

It has been speculated that intensification of camel herding in the Arabian Peninsula has increased the virus

Published online: 29 June 2016

reproductive number and attack rate in camel herds, while the 'urbanization' of camel herding increased the frequency of zoonotic 'spillover' infections from camels to humans [8].The current control measures of MERS-CoV infection in camel include control of camel movement where camels with detectable MERS-CoV RNA at borders are quarantined and tested at regular intervals, the use of personal protective equipment's while handling camels, awareness campaigns on the risks of consuming unpasteurized camel milk and urine, and targeting camel owners and the general public [9].

To date, no vaccine or specific treatment for MERS-CoV is available. However, a recent orthopoxvirus-based vaccine against MERS-CoV infection in camels has been developed. Vaccination of dromedary camels with this vaccine induced protective immunity resulting in reduction of excreted infectious MERS-CoV, without an evidence for antibody-dependent enhancement of viral replication [10].

In terms of the number of cases of MERS-CoV infection among humans in various countries, the UAE ranks third after the Kingdom of Saudi Arabia and South Korea [5]. The UAE also has a continuous movement of camels to and from neighboring countries, including the Kingdom of Saudi Arabia, Oman, and Qatar. A previous report has shown that MERS-CoV genome was detected in camels nasal swabs obtained from different locations including slaughter houses located within the Abu Dhabi Emirate or from borders with Saudi Arabia and Oman. High infection rate was observed in camels at slaughter houses when compared to borders with Saudi Arabia and Oman, whereas no virus was detected in public escorts and zoos [11].

To date, there is only one reported MERS-CoV human infection (http://www.haad.ae/haad/tabid/58/ctl/Details/Mid/417/ItemID/487/Default.aspx) in the UAE with a strong evidence of connection with infected dromedary camels imported from Oman [12]. However, the complete or partial characterization of MERS-CoV genome in camels located in farms linked with human infection has not been reported, to our knowledge. In this study, we conducted a systemic surveillance of MERS-CoV in camel farms in response to human infection notifications where the patient had a history of contact with animals. The prevalence of MERS-CoV and the possible transmission of the virus to humans in Abu Dhabi Emirate were investigated.

Materials and methods

Samples

This study was performed between February and May 2014 at the Abu Dhabi Food Control Authority Veterinary Laboratory, Abu Dhabi, UAE. It was a part of an epidemiological investigation response to three separate notifications issued by the Health Authority, Abu Dhabi, UAE (http://www.who.int/csr/don/2014_03_12/en/;http://www.who.int/csr/don/2014_02_07mers/en/;http://www.who.int/csr/don/2014_04_10_mers/en/) of MERS-CoV human infection who had a history of contact with animals.

A total of 1147 nasal swabs were obtained from all animals in 39 camel farms and 1 sheep farm. These nasal swabs included those collected from animals in farms (A, B, and C) that belonged to the 3 infected humans. Farms A, B, and C had 75 camels, 46 camels, and 34 sheep, respectively. Farm A was located in the Western region of the Abu Dhabi Emirate (Gayathi), whereas Farms B and C were located in the Eastern region. In addition to the nasal swabs from Farms A, B, and C, 992 nasal swabs were collected from all camels in 37 farms which were located within a 3-km-radius zone around Farm B. Farms A and C were not surrounded by camel farms within a 3-km-radius zone. A sputum sample from each infected farm owner was also collected.

On farms with MERS-CoV-positive animals, samples of drinking water (10) available for laborers and animals were collected and tested for the presence of the virus. To investigate whether milk suckling played a role in viral transmission, the available milk samples (15) from dams of 19 MERS-CoV-positive calves (<1 year old), were tested. Furthermore, in order to follow-up viral shedding, both nasal and rectal swabs from these MERS-CoV-positive calves and their corresponding dams were subsequently tested on each of the 4 occasions (at 1-week intervals) after the initial virus detection. In parallel, a total of 16 sputum samples collected from laborers working in camels farms (A, B and C) linked with the MERS-CoV human notifications were also tested.

All nasal swabs from animals were transferred to the veterinary laboratory in a universal transport medium within 24 h after collection. Sputum samples obtained from the infected farm owners and laborers were tested in Sheikh Khalifa Medical City, Abu Dhabi, UAE.

Personal protective equipment including N95 masks, goggles, disposable gowns, gloves, and head covers was used during sample collection, transport, and testing. The study was approved by the Abu Dhabi Health Authority and Abu Dhabi Food Control Authority ethical committees.

Nucleic acid extraction, PCR procedures, and sequencing

The RNA was extracted from nasal swabs and sputum samples using Qiagen viral RNA extraction kit, according to the manufacturer's instructions (Qiagen GmbH, Hilden, Germany). Two published MERS-CoV RT-qPCR assays

were performed; one was used as a screening assay targeting upstream of the E protein gene (UpE) and the other was used as a confirmation assay (targeting the ORF1a gene region). The ORF1b gene region was also tested before being replaced by Orf1a [13, 14]. All assays were optimized by the use of a real-time 1-step RT-qPCR kit for analysis of viral RNA and cycling system (Roche Diagnostics). Positive results for both UpE and ORF1a assays were further confirmed by sequencing the RNA-dependent RNA polymerase and nucleocapsid gene regions as well as sequencing 5 partial fragments (ORF1a, ORF1ab, Spike1, Spike2, and ORF4b genes) as previously described [15].

Analysis of sequence data

Phylogenetic analysis on the basis of ORF1a, ORF1ab, Spike1, Spike2, and ORF4b concatenated partial genes sequences of MERS-CoV sequences from 3 UAE camels characterized in this study (camels 1 from farm B and camels 9,11 from farm A) and 1 human complete genome previously reported (farm A owner from Gayathi) [16] (accession numbers are as follows: Camel1ORF1a:KP202190; Camel1ORF1ab:KP202202; Camel1Spike1:KP202214; Camel1Spike2:KP202226; Camel1ORF4b:KP202238; Camel9ORF1a:KP202195; Camel9ORF1ab:KP202207; Camel9Spike1:KP202219; Camel9Spike2:KP202231; Camel9 ORF 4b:KP202243; Camel11bORF1a:KP202197; Camel11bORF1ab:KP202209; Camel11bSpike1:KP202221; Camel11bSpike2:KP202233; Camel11b ORF 4b:KP202245 and KP209310) together with published camel ($n = 17$), and human (48) MERS-CoV sequences were carried out with MEGA5 version 5. The evolutionary distances were estimated by means of the maximum likelihood method based on the Kimura 2-parameter model. Bootstrap analyses were performed with 1000 repeat samples of the data sets [17].

Results

Outbreak investigation

In total, 1147 nasal swabs were collected from 1147 animals living in 39 camel farms and 1 sheep farm. Among the 40 farms screened, MERS-CoV was detected by RT-qPCR assays in 6 (15 %) camel farms; the virus was not detected in any of the 34 nasal swabs collected from the single sheep farm (Farm C; Table 1). The 6 MERS-CoV-positive farms were Farms A and B (owned by 2 of the 3 infected farm owners) together with 4 other camel farms (designated as D, E, F, and G) located in a 500-m-radius region surrounding farm B in the eastern region. There were 324 camels on these 6 farms.

Overall, 42 of the 1147 (3.7 %) nasal swabs yielded positive results. The total numbers of swabs collected from farms A, B, D E, F, and G were 75, 47, 25, 36, 111, and 30, respectively, of which 9 (12.0 %), 6 (12.8 %), 1 (4.0 %), 1 (2.8 %), 2 (1.8 %), and 23 (76.6 %), respectively, were MERS-CoV positive. Of the 42 MERS-CoV-positive camels, 16 (38.1 %) were <1 year old and 26 (61.9 %) were > 2 years old; 25 were female and 17 were male (Table 1). Farms D E, F, and G were located within a 500-m-radius zone surrounding farm (B).

Nasal swabs were collected from the MERS-CoV-positive camels on 4 subsequent occasions (at 1-week intervals) for testing. One week after the initial testing, the virus was detected in 2 camels (1 each in farm B and F). Two weeks after the initial testing, the virus was detected in those 2 camels and 1 other camel in farm A. All 3 camels were negative for the virus 3 weeks after the time of the first virus detection. In contrast, the virus was not detected on the other farms (D, E, and G) in any of the 4 subsequent tests. All water and milk samples collected from the 6 infected camel farms were negative for MERS-CoV. However, the virus was detected in 1 rectal swab obtained from a camel in farm A 1 week after the initial testing; in the 3 subsequent tests, results for this camel were negative.

All sputum samples from the 3 farm owners were positive for MERS-CoV, as determined by RT-qPCR assays. The owner of farm A was a 68-year-old man with a history of repeated visits to his farm, direct exposure to the camels, and recurrent drinking of raw camel milk. In addition, this patient reported recent travel to Oman. The owner of farm B was a 66-year-old man, who visited his farm, had direct exposure to the camels, and drank raw camel milk on daily basis. The owner of farm C, which contained sheep, was a 64-year-old man. He had a history of travel to the Kingdom of Saudi Arabia, where he was exposed to camels within 14 days prior to testing positive for MERS-CoV. All sputum samples (16) collected from labors of farms A, B, and C linked with MERS-CoV human notifications were negative for MERS-CoV. Similarly, MERS-CoV was not detected in other 3, 3, 11, and 10 sputum samples collected from labors of farms D, E, F, and G detected positive MERS-CoV.

Sequences and phylogenetic analysis

The two camels (9 and 11) concatenated (ORF1a, ORF1ab, Spike1, Spike2, and ORF4b genes) partial sequences derived from Farm A were almost identical to each other and exhibited 99.9 % identity (differing in 6 of 4184 nts) to the UAE-Abudhabi/Gayathi sequence derived from human linked with the same farm. Sequencing of other viral genes

Table 1 Age and sex distribution of MERS-CoV-positive animals in 6 dromedary camel farms (A, B, D, E, F, and G) and 1 sheep farm (C)

Farm	Species	No. of linked human cases of infection	Total no of animals	No. (%) of MERS-CoV-positive animals	Age of MERS-CoV-positive animals		Sex of MERS-CoV-positive animals	
					<1 year	>2 year	F	M
A	Camel	1	75	9 (12.0)	8	1	5	4
B	Camel	1	47	6 (12.8)	6	0	3	3
C	Sheep	1	34	0 (0)	0	0	0	0
D	Camel	0	25	1 (4.0)	1	0	1	0
E	Camel	0	36	1 (2.8)	1	0	1	0
F	Camel	0	111	2 (1.8)	0	2	2	0
G	Camel	0	30	23 (76.7)	0	23	13	10
Total		3	358	42 (11.7)	16	26	25	17

detected from the rest of camels and humans was not successful.

Phylogenetic analysis involving the maximum likelihood algorithm revealed that MERS-CoV-published sequences derived from camels and a human were grouped in 2 main clusters (A and B) separated by a 94-bootstrap value. The sequences for the 3 UAE camels (camel 1, camel 9, camel 11) and 1 human (Farm A owner from Gayathi) together with other camels' MERS-CoV sequences reported before from the UAE formed a separate cluster located within the large (A) cluster of human and camel MERS-CoV sequences reported for neighboring countries including Kingdom of Saudi Arabia and Qatar (Fig. 1).

Discussion

Dromedary camels may act as a direct source for human MERS-CoV infection [18]. Widespread distribution of MERS-CoV-neutralizing antibodies in dromedary camels in the UAE has been revealed by analyzing the serum samples previously collected in 2003 and 2005 [19, 20]. Later, the molecular detection of the virus in dromedary camels has been reported in racing, breeding and dairy farms, camels' slaughter houses, and at the borders with Saudi Arabia and Oman [11, 21]. However, to date, no study has been published targeting the detection of the MERS-CoV genome in camel farms associated with human infection in the country. The present study is believed to be the first report on the partial characterization of MERS-CoV genome from camels located in farms linked with human infection in Abu Dhabi Emirate.

In the present study, results revealed that MERS-CoV genome was detected in 6 (15 %) of the 40 farms tested. For the 1147 nasal swabs from 1147 camels and sheep, the prevalence of MERS-CoV was found to be 3.7 %. Such prevalence is higher when compared to the previously reported 1.6 % molecular prevalence detected in 7803 camels' nasal swabs, where positive camels were detected at the borders with Saudi Arabia and Oman and in camels' slaughter houses suggesting that such locations represent higher hot spots for potential human and animal infections [11]. However, the observed high prevalence reported here in camel's farms not subjected to animal movements points out that such farms are actively participating in the spread of the virus among animals or humans. Thus, it is necessary to investigate such farms when conducting systematic surveillances or prior to establishing disease control programs.

The prevalence of MERS-CoV in infected camel farms varied from 1.8 % in farms F and 76.7 % in farm G. Studies of MERS-CoV RNA prevalence in nasal swab specimens from dromedaries in the field or abattoirs have found rates of 0–15 % (by PCR) among adults and 35 % among calves [3, 22].The high prevalence in farm G could be attributed to active circulation of the virus in the herd where both calves and adults can be infected. This suggests that prior infection or passively acquired maternal antibody might not provide complete protection from infection [23].

In the present study, analysis of both nasal and rectal swabs revealed viral shedding from infected camels for up to a maximum duration of 2 weeks after the first detection time. In contrast, MERS-CoV was not detected in milk and water samples collected from infected farms. Variable durations (28 and 35 days) of viral RNA detection after infection both in field and experimental studies together with the absence of detectable virus in milk and water samples have been previously reported [2]. The detection of MERS-CoV RNA in camels with pre-existing antibodies

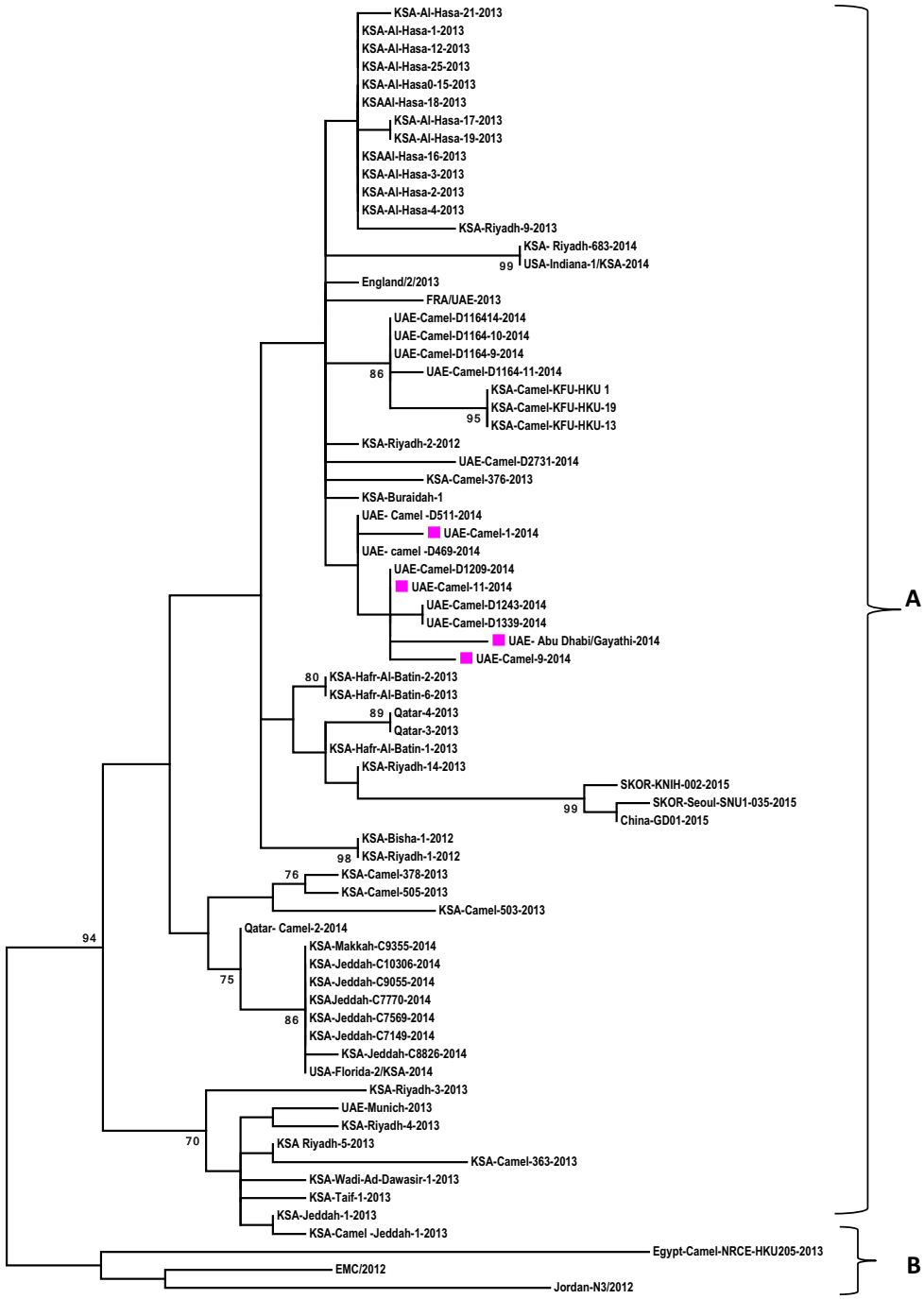

Fig. 1 Phylogenetic analysis of new ORF1a, ORF1ab, Spike1, Spike2, and NSP4 partial MERS-CoV nucleic acid sequences from 1 human and 3 camels in the UAE, together with corresponding published MERS-CoV sequences (available in GenBank) from 17 camels and 48 humans. Values ≥70 are indicated on the branches as percentages. Sequences from GenBank are identified by the source country followed by the name of the strain. Sequences from the present study (pink rectangles) are named as follows: UAE-Camel, UAE-Abudhabi/Gayathi. The two major groups were identified as A, B. The evolutionary distances were estimated my means of the maximum likelihood method (based on the Kimura 2-parameter model). Bootstrap analyses were performed with 1000 repeat samples of the data sets. *EMC* Erasmus Medical Center, *FRA* France, *KSA* Kingdom of Saudi Arabia, *SKOR* South Korea

in a herd in KSA together with the lack of correlation between viral RNA loads and levels of neutralizing antibodies in camels for slaughter in Qatar indicated a limited immune protection and a potential for reinfection despite previous exposure [23, 24] Indeed, circulation up to 2–4 months of the same virus strains at the herd level has been demonstrated in other studies [21, 24]. The detection of MERS-CoV in rectal swabs in the present study may suggest another source of transmission to animals and humans [25].

The presence of MERS-CoV in 4 camel farms (D, E, F, and G) located within a 500-m-radius zone surrounding a MERS-CoV-positive farm (B) suggests that direct, aerosol, or fomite transmission could be the route of virus spread among camel farms [26]. However, further investigation is needed to exclude the possibility that these farms became infected independently of farm B. It should be noted that MERS-CoV-positive farm A was not surrounded by other farms within a 3-km-radius zone, and hence no near neighbor farms were available for screening. The fact that

MERS-CoV genome was not detected in another species (i.e., sheep) in the present study is in accordance with previous reports [25].

Interestingly, MERS-CoV genome was not detected in nasal swabs collected from laborers working in MERS-CoV-positive camel farms. Early MERS-CoV antibody response was associated with reduced disease severity in human [27]. In contrast, it has been reported that none of the laborers who were in daily contact with MERS-CoV-positive camel had serologic evidence of infection. Accordingly, it was concluded that MERS-CoV was not highly transmissible from dromedaries to humans with various levels of exposure to this infected dromedary herd [28].This finding necessitates further studies on the mechanisms by which MERS-CoV is transmitted from dromedaries to humans and the heterogeneity of human susceptibility to this virus are needed.

The MERS-CoV-positive camel farms included 2 premises (farms A and B) that were linked to 2 reported human cases. Virus detection in camel farms linked with infected humans has been reported previously [2] suggesting that cross-species transmission may occur and that camels may act as a direct source of MERS-CoV infection in humans.

The 2 infected owners of farms A and B had histories of repeated visits to these farms and drinking raw camel milk. They were not in contact with other confirmed human cases of MERS-CoV infection. During the outbreak investigation, it was found that both farms A and B had no animal movement or introduction of new animals to the herds for at least 1 month prior to the reports of the 2 cases. It has been reported that camels are likely to be a major reservoir host for MERS-CoV and that infection with this virus among camels on the Arabian Peninsula seems to be common [3]. This indicated that the 2 farm owners may have acquired viral infection through aerosols, fomites, or direct contact with camels. However, the exact route of human or camel infection and whether camels in both farms were previously infected remains unknown.

The 3 UAE partial (4.2-kb) camel sequences characterized in this study and 1 human concatenated sequences together with other camels sequences reported from the UAE formed a separate cluster located within the large cluster containing human and camel MERS-CoV sequences reported in the neighboring countries including the Kingdom of Saudi Arabia and Qatar. Furthermore, two camels partial sequences derived from camel farm exhibited 99.9 % identity with the corresponding sequences derived from human who had a history of contact with camels in the same farm. This has provided evidence of potential zoonosis and suggests a common origin.

In conclusion, the molecular detection of MERS-CoV in camel farms linked with human infections in the UAE has indicated that camels may have a role in the transmission of the virus to humans. However, for further understanding of the epidemiology of MERS-CoV in the UAE, more complete genome sequences from both human and camels are required.

Compliance with ethical standards

Conflict of Interest Authors declare that they have no conflict of interest.

Ethical approval The study was approved by the Abu Dhabi Health Authority and Abu Dhabi Food Control Authority ethical committees.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. A.M. Zaki, S. van Boheemen, T.M. Bestebroer et al., Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N. Engl. J. Med. **367**, 1814–1820 (2012)
2. E.I. Azhar, S.A. El-Kafrawy, S.A. Farraj et al., Evidence for camel-to-human transmission of MERS coronavirus. N. Engl. J. Med. **370**, 2499–2505 (2014)
3. N. Nowotny, J. Kolodziejek, Middle East respiratory syndrome coronavirus (MERS-CoV) in dromedary camels, Oman, 2013. Euro. Surveill. **19**(16), 20781 (2014)
4. B.L. Haagmans, S.H. Al Dhahiry, C.B. Reusken et al., Middle East respiratory syndrome coronavirus in dromedary camels: an outbreak investigation. Lancet Infect. Dis. **14**, 140–145 (2014)
5. WHO Middle Respiratory Syndrome Coronavirus update http://www.who.int/csr/don/26-march-2016-mers-saudi-arabia/en/
6. L. Du, G.Z. Han, Deciphering MERS-CoV evolution in dromedary camels. Trends Microbiol. **24**(2), 87–89 (2016). doi:10.1016/j.tim.2015.12.013
7. C.B. Reusken, V.S. Raj, M.P. Koopmans, B.L. Haagmans, Cross host transmission in the emergence of MERS coronavirus. Curr. Opin. Virol. **16**, 55–62 (2016). doi:10.1016/j.coviro.2016.01.004
8. C. Gossner, N. Danielson, A. Gervelmeyer, F. Berthe, B. Faye, K. Kaasik Aaslav, C. Adlhoch, H. Zeller, P. Penttinen, D. Coulombier, Human-dromedary camel interactions and the risk of acquiring zoonotic Middle East respiratory syndrome coronavirus infection. Zoonoses Public Health. **63**(1), 1–9 (2016). doi:10.1111/zph.12171
9. A.S. Omrani, J.A. Al-Tawfiq, Z.A. Memish, Middle East respiratory syndrome coronavirus (MERS-CoV): animal to human interaction. Pathog. Glob. Health. **109**(8), 354–362 (2015). doi:10.1080/20477724.2015
10. B.L. Haagmans, J.M. van den Brand, V.S. Raj, A. Volz, P. Wohlsein, S.L. Smits, D. Schipper, T.M. Bestebroer, N. Okba, R. Fux, A. Bensaid, D. Solanes Foz, T. Kuiken, W. Baumgärtner, J. Segalés, G. Sutter, A.D. Osterhaus, An orthopoxvirus-based vaccine reduces virus excretion after MERS-CoV infection in dromedary camels. Science. **351**(6268), 77–81 (2016). doi:10.1126/science.aad1283
11. M.F. Yusof, Y.M. Eltahir, W.S. Serhan, F.M. Hashem, E.A. Elsayed, B.A. Marzoug, A.S. Abdelazim, O.K. Bensalah, S.S. Al Muhairi, Prevalence of Middle East respiratory syndrome coronavirus (MERS-CoV) in dromedary camels in Abu Dhabi Emirate, United Arab Emirates. Virus Genes. **50**(3), 509–513 (2015)
12. Z.M. Al Hammadi, D.K. Chu, Y.M. Eltahir, F. Al Hosani, M. Al Mulla, W. Tarnini, A.J. Hall, R.A. Perera, M.M. Abdelkhalek,

J.S. Peiris, S.S. Al Muhairi, L.L. Poon, Asymptomatic MERS-CoV infection in humans possibly linked to infected dromedaries imported from Oman to United Arab Emirates, May 2015. Emerg Infect Dis. **21**(12), 2197–2200 (2015)
13. Corman VM, Eckerle I, Bleicker T et al a. Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. Euro. Surveill. **17**(39), pii: 20285 (2012)
14. Corman VM, Müller MA, Costabel U et al Assays for laboratory confirmation of novel human coronavirus (hCoV-EMC) infections. Euro. Surveill. **17**(49), pii: 20334 (2012)
15. Smits Saskia L, Raj VStalin, Pas Suzan D, et al. Reliable typing of MERS-CoV variants with a small genome fragment. J. Clin. Virol. http://dx.doi.org/10.1016/j.jcv.2014.12.006
16. J.C. Hunter, D. Nguyen, B. Aden, Z. Al Bandar, W. Al Dhaheri, K. Abu Elkheir, A. Khudair, M. Al Mulla, F. El Saleh, H. Imambaccus, N. Al Kaabi, F.A. Sheikh, J. Sasse, A. Turner, L. Abdel Wareth, S. Weber, A. Al Ameri, W. Abu Amer, N.N. Alami, S. Bunga, L.M. Haynes, A.J. Hall, A.J. Kallen, D. Kuhar, H. Pham, K. Pringle, S. Tong, B.L. Whitaker, S.I. Gerber, F.I. Al Hosani, Transmission of Middle East Respiratory Syndrome Coronavirus Infections in Healthcare Settings, Abu Dhabi. Emerg. Infect. Dis. **22**(4), 647–656 (2016). doi:10.3201/eid2204.151615
17. K. Tamura, D. Peterson, N. Peterson et al., MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. **28**, 2731 (2011)
18. Z.A. Memish, M. Cotton, B. Meyer et al., Human infection with MERS coronavirus after exposure to infected camels, Saudi Arabia, 2013. Emerg. Infect. Dis. (2014). doi:10.3201/eid2006.140402
19. B. Meyer, M.A. Müller, V.M. Corman et al., Antibodies against MERS coronavirus in dromedary camels, United Arab Emirates, 2003 and 2013. Emerg. Infect. Dis. **20**, 552–559 (2014)
20. S. Alexandersen, G.P. Kobinger, G. Soule, Middle East respiratory syndrome coronavirus antibody reactors among camels in Dubai, United Arab Emirates, in 2005. Transbound. Emerg. Dis. **61**, 105–110 (2014)
21. U. Wernery, V.M. Corman, E.Y. Wong, A.K. Tsang, D. Muth, S.K. Lau, K. Khazanehdari, F. Zirkel, M. Ali, P. Nagy, J. Juhasz, R. Wernery, S. Joseph, G. Syriac, S.K. Elizabeth, N.A. Patteril, P.C. Woo, C. Drosten, Acute middle East respiratory syndrome coronavirus infection in livestock Dromedaries, Dubai, 2014. Emerg. Infect. Dis. **21**(6), 1019–1022 (2015)
22. D.K. Chu, L.L. Poon, M.M. Gomaa, M.M. Shehata, R.A. Perera, D. Abu Zeid et al., MERS coronaviruses in dromedary camels. Egypt. Emerg. Infect. Dis. **20**, 1049–1053 (2014). doi:10.3201/eid2006.140299
23. M.G. Hemida, D.K.W. Chu, L.L.M. Poon, R.A.P.M. Perera, M.A. Alhammadi, H.-Y. Ng et al., MERS coronavirus in dromedary camel herd. Saud. Arab. Emerg. Infect. Dis. **20**(7), 1231–1234 (2014). doi:10.3201/eid2007.140571
24. E. Farag, C. Reusken, B. Haagmans, K. Mohran, V.S. Raj, S. Pas, J. Voermans, S.L. Smits, G.J. Godeke, M.M. Alhajri et al., High proportion of MERS-CoV shedding dromedaries at slaughterhouse with potential epidemiological link to human cases, Qatar 2014. Infect. Ecol. Epidemiol. **5**, 28305 (2015)
25. D.R. Adney, N. van Doremalen, V.R. Brown et al., Replication and shedding of MERS-CoV in upper respiratory tract of inoculated dromedary camels. Emerg. Infect. Dis. (2014). doi:10.3201/eid2012.141280
26. A.N. Alagaili, T. Briese, N. Mishra et al., Middle East respiratory syndrome coronavirus infection in dromedary camels in Saudi Arabia. mBio **5**, e00884–e008814 (2014). doi:10.1128/mBio.00884-14
27. W.B. Park, R.A. Perera, P.G. Choe, E.H. Lau, S.J. Choi, J.Y. Chun, H.S. Oh, K.H. Song, J.H. Bang, E.S. Kim, H.B. Kim, S.W. Park, N.J. Kim, L.L. Man Poon, M. Peiris, M.D. Oh, Kinetics of serologic responses to MERS coronavirus infection in humans, South Korea. Emerg. Infect. Dis. **21**(12), 2186–2189 (2015). doi:10.3201/eid2112.151421
28. M.G. Hemida, A. Al-Naeem, R.A. Perera, A.W. Chin, L.L. Poon, M. Peiris, Lack of middle East respiratory syndrome coronavirus transmission from infected camels. Emerg. Infect. Dis. **21**(4), 699–701 (2015). doi:10.3201/eid2104.141949