

Middle East respiratory syndrome coronavirus (MERS-CoV) in dromedary camels, Oman, 2013

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A countrywide survey in Oman revealed Middle East respiratory syndrome coronavirus (MERS-CoV) nucleic acid in five of 76 dromedary camels. Camel-derived MERS-CoV sequences (3,754 nucleotides assembled from partial sequences of the open reading frame (ORF)1a, spike, and ORF4b genes) from Oman and Qatar were slightly different from each other, but closely related to human MERS-CoV sequences from the same geographical areas, suggesting local zoonotic transmission. High viral loads in nasal and conjunctival swabs suggest possible transmission by the respiratory route.

Background

In June 2012 a novel betacoronavirus, subsequently named Middle East respiratory syndrome coronavirus (MERS-CoV), was isolated from a patient with fever and respiratory symptoms who had been admitted to a hospital in Jeddah, Saudi Arabia [1]. As of 22 April 2014, the number of reported laboratory-confirmed cases of MERS-CoV worldwide amounts to 333, with 107 deaths [2]. To date, only fifteen cases, including ten in Europe, have been reported outside of the Middle East [2] and the vast majority of cases were reported from Saudi Arabia. Thus, it seems that the virus may originate from the Arabian Peninsula. Family, healthcare associated and community case clusters of MERS-CoV infections have been reported (e.g. [3]).

Besides limited human-to-human transmission, however, epidemiological data point towards an animal reservoir of MERS-CoV. First evidence of such a reservoir host was provided when all 50 investigated sera of dromedary camels (*Camelus dromedaries*) from Oman exhibited high-titre neutralising antibodies against MERS-CoV [4]. This observation has meanwhile been confirmed by several studies from other countries on the Arabian Peninsula and beyond (e.g. Egypt [5]). Other important farm animals on the Arabian Peninsula such as cattle, goats, sheep and chickens were also investigated but were found negative [6,7]. Although different species of bats carry a variety of coronaviruses and

have been suggested as the most likely primary animal reservoir for MERS-CoV, so far only a short (190 nucleotides (nt)) sequence in a conserved region of the MERS-CoV genome was amplified from a faecal pellet of a bat (*Taphozous perforates*) in Saudi Arabia [8]. The present study was initiated in order to identify the virus in camels and to compare it genetically with human-derived MERS-CoV.

Sample collection

In December 2013, nasal and conjunctival swabs were taken from 76 dromedary camels of different age, breed and sex from all over Oman. All swabs were taken in duplicates, one stored in virus isolation medium and the other in the virus-inactivating buffer DNA/RNA Shield (Zymo Research, Irvine, USA). The former samples were frozen at -80°C at the Veterinary Research Center of the Ministry of Agriculture and Fisheries, Oman, for later use, while the latter samples were shipped to the University of Veterinary Medicine Vienna for analysis. DNA/RNA Shield effectively lyses cells and inactivates nucleases and infectious agents. In addition it ensures nucleic acid stability during sample storage and transport at ambient temperatures.

Nucleic acid extraction and polymerase chain reactions

RNA was extracted employing a Quick-RNA MiniPrep Kit (Zymo Research, Irvine, USA), following the manufacturer's instructions.

For screening, two published MERS-CoV reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assays were performed, one in the open reading frame (ORF)1a gene region [9] and the other in the ORF1b gene region [10], and both were optimised for SuperScript III Platinum One-Step RT-qPCR System (Invitrogen/LifeTechnologies, CA, USA). For confirmation, the positive samples were retested by reverse transcription-polymerase chain reactions (RT-PCRs) in the RNA-dependent RNA polymerase (RdRp)- and nucleocapsid (N) gene regions [9] as well as in the spike

TABLE

Primers used for amplification of Middle East respiratory syndrome coronavirus genetic sequences and subsequent phylogenetic analysis, 2013

Primer name, position ^a and sequence	Target sequence	Expected size
MERS_1767_F: 5'-CTCGCAATTCTCTGGAAC-3' MERS_2615_R: 5'-GTCAGTAGGTTGGAGCAGTC-3'	ORF1a	848 nt
MERS_11419_F: 5'-CAA GCC CCA TTG CCT ATC TG-3' MERS_12064_R: 5'-GCT TGA AGT ACG CTA GGA GTG-3'	ORF1a	645 nt
MERS_22074_F: 5'-CGTAATGCCAGTCTGAACTC-3' MERS_23127_R: 5'-CAGGGTGAGTATTGATTAGCG-3'	Spike	958 nt
MERS_24156_F: 5'-GCTGATCCTGGTTATATGCAAGG-3' MERS_24903_R: 5'-CAACCTCAATGTGGTTGCTAGG-3'	Spike	747 nt
MERS_26042_F: 5'-CTT TGG CCA AAC AGG ACG CA-3' MERS_26856_R: 5'-GAC GCC GAG AAA GCC ATA GTT C-3'	ORF4b	814 nt

F: forward; MERS: Middle East respiratory syndrome; nt: nucleotide; ORF: open reading frame; R: reverse.

^a Referring to the sequence of the MERS-coronavirus strain HCoV-EMC/2012 with the GenBank accession number: JX869059.

gene region [11] and by five other RT-PCRs designed for subsequent phylogenetic analysis (Table). The MERS-CoV genome organisation and the location of the five RT-PCR amplicons used to generate a concatenated sequence for phylogenetic analysis are displayed in Figure 1.

All conventional RT-PCRs were conducted using OneStep RT-PCR kit (Qiagen, Hilden, Germany). Primer synthesis and sequencing in both directions were carried out by Microsynth (Balgach, Switzerland). The obtained sequences were verified by Basic Local Alignment Search Tool (BLAST) search, aligned using the ALIGN PLUS programme (Scientific & Educational Software), and compiled to one concatenated sequence.

Phylogenetic analysis

For phylogenetic analysis, three camel-derived (from Oman, Qatar, and Egypt) and 33 human-derived MERS-CoV sequences were included. A multiple sequence alignment was performed using BioEdit Sequence Alignment Editor version 7.0.9.0 and verified using the CLUSTAL_X programme (version 1.8). Phylogenetic neighbour-joining and maximum likelihood analyses were conducted

with the help of the Molecular Evolutionary Genetics Analysis (MEGA) 5 programme [12]. The evolutionary distances were computed using the Kimura 2-parameter model. Bootstrap resampling analysis with 1,000 replicates was employed. The sequences used for phylogenetic analysis were deposited at GenBank under the accession numbers KJ573789–KJ573793 (MERS-CoV sequences derived from camel Oman_30_2013), and KJ598493–KJ598496 and KF933384 (sequences derived from camel Qatar_1_2013).

Results

Nasal and conjunctival swabs of five of a total of 76 camels (6.6%) proved positive in all applied RT-qPCR and RT-PCR assays. The cycle threshold (Ct) values ranged from 15.74 to 36.29. Concatenated sequences of a total of 3,754 nts were obtained from all five positive Omani camels by compiling the sequences derived from the five different PCR amplification products covering the ORF1a, spike and ORF4b gene regions (Table, Figure 1). The five concatenated sequences derived from the Omani camels were 100% identical to each other, and they exhibited 99% identity (differing in 12 of 3,754 nts) to the sequence derived from the Qatari

FIGURE 1

Genome organisation of Middle East respiratory syndrome coronavirus and location of five reverse transcription-polymerase chain reaction amplification products used to generate a concatenated sequence for phylogenetic analysis, 2013

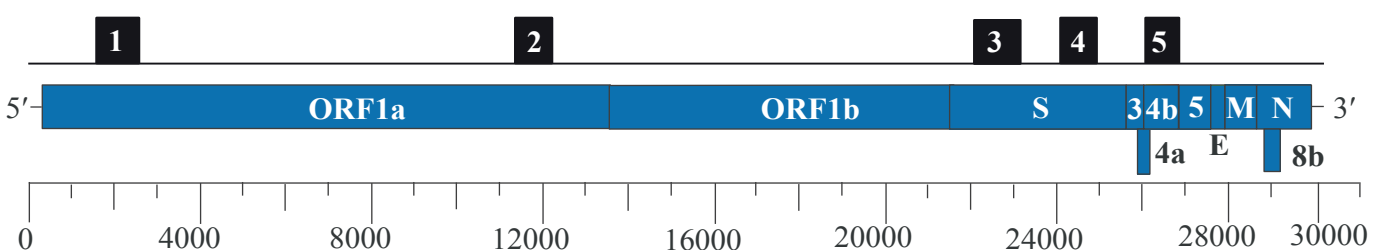
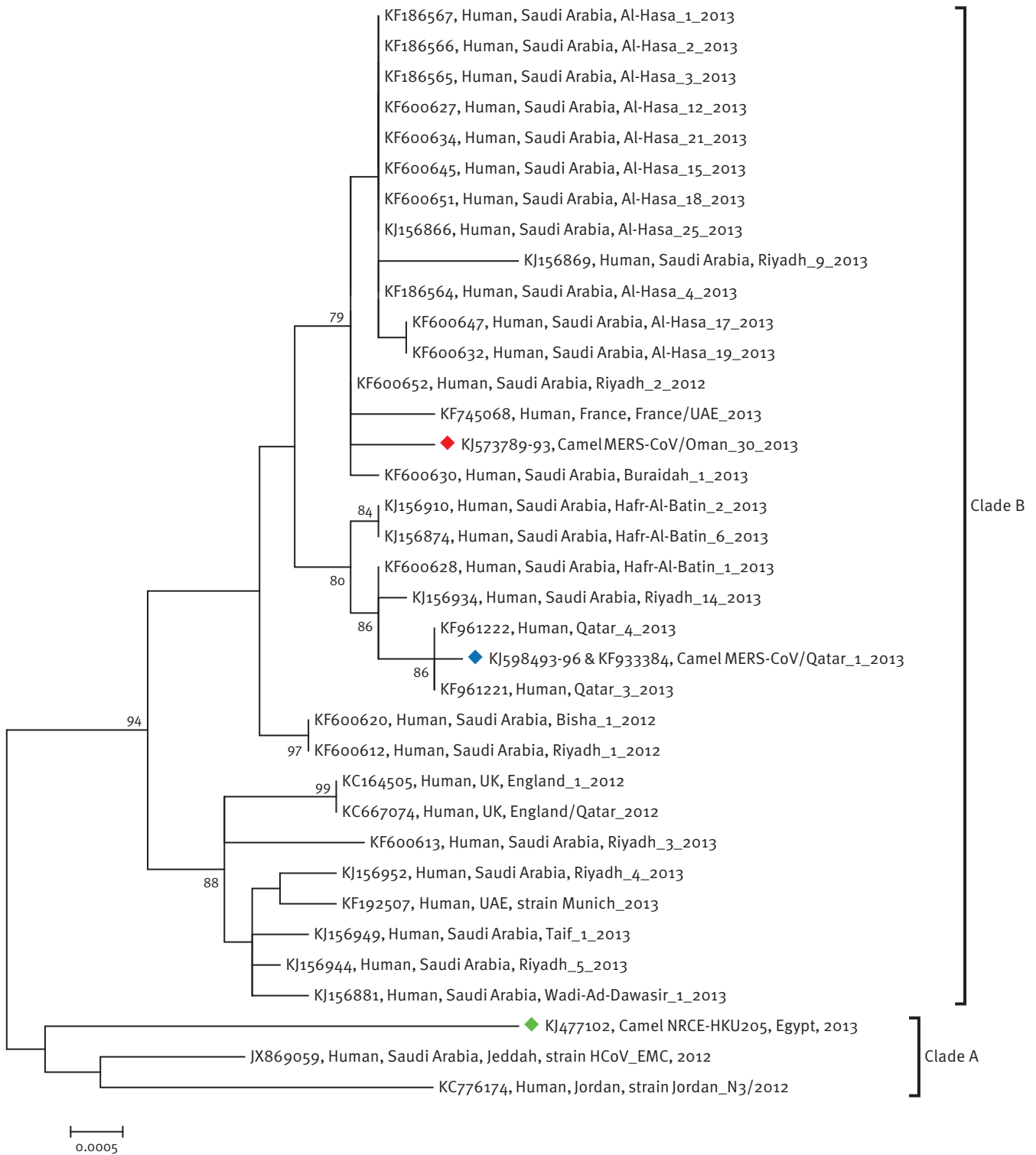


FIGURE 2

Phylogenetic analysis of three camel- and 33 human-derived Middle East respiratory syndrome coronavirus (MERS-CoV) nucleotide sequences, 2013



UAE: United Arab Emirates; UK: United Kingdom.

Each 3,754 nucleotide long sequence used to generate the tree was obtained from concatenating partial sequences of the open reading frame (ORF)1a, spike and ORF4b gene regions. Of note the different clustering of the camel-derived sequences originating from Oman (marked with a red diamond), Qatar (blue diamond) and Egypt (green diamond). The Qatari and Omani camel-derived MERS-CoV sequences cluster close to the human-derived sequences originating from the same areas.

camel and also 99% identity (31 nts difference) to the known respective sequence derived from an Egyptian camel (Figure 2).

The phylogenetic analysis involving all three currently available camel-derived MERS-CoV sequences and all 33 available human-derived sequences is shown in Figure 2. It clearly indicates that the camel-derived MERS-CoV sequences are clustering independently from each other, but together with the human-derived MERS-CoV sequences from the same geographical areas. This could be demonstrated unambiguously for the Qatari samples (exhibiting only 1 nt difference between the camel-derived and the human-derived MERS-CoV sequences), but also for the camel-derived Omani sequence, which is clustering close to a human MERS-CoV sequence from neighbouring United Arab Emirates (GenBank accession number: KF745068, with only 5 of 3,754 nts being different; no human Omani MERS-CoV sequences are available so far). For the Egyptian camel-derived MERS-CoV sequence [13] no human-derived sequence with as high relative sequence identity and/or from a respectively close geographical area has yet been detected.

Discussion

The results of our study and similar studies from Qatar [11] and Saudi Arabia [14-16] show a close genetic relationship between camel-derived and human-derived MERS-CoV from the same geographical areas, suggesting local zoonotic transmission. A proof of cross-species transmission of MERS-CoV from dromedary camels to humans was reported in a publication on human infection with MERS-CoV after exposure to infected camels in Saudi Arabia recently [15]. The authors concluded that camels may act as a direct source of human MERS-CoV infection. For the implementation of effective, but reasonable precautions, however, further pieces of the puzzle must be put together: a Ct-value of 15.74, corresponding to approximately 33 million RNA copies, in the nasal swab of one of the Omani camels (this study) indicates a very high virus load and the probability of the transmission of high numbers of virus particles by the nasal route. Data from another recent publication suggest that neither passively acquired maternal antibodies nor prior infections seem to lead to full immunity in the camels, and viral nucleic acid was detected in such animals, though at a significantly lower level [16]; these and other recent results, however, require confirmation.

Evidence was provided that MERS-CoV has been circulating in camels at least since 1992 [14], but probably much longer, while strains in humans emerged only recently [17]. Initially, dromedary camels have been considered possible intermediate hosts of MERS-CoV, while bats were suggested as primary reservoirs – in analogy to severe acute respiratory syndrome-coronavirus (SARS-CoV). According to our current knowledge, however, dromedary camels might even be considered the primary reservoir hosts of MERS-CoV. Comparative

analyses of camel- and human-derived MERS-CoV sequences exhibit >99% nt identities to each other with so far no striking genetic differences observed. Given the high prevalence of MERS-CoV-specific antibodies in dromedary camels [4,5], infection of camels with this virus seems to be frequent on the Arabian Peninsula. This is in sharp contrast to the comparatively low number of recorded human infections. Since currently no other obvious explanation for this discrepancy exists, it is postulated that a high infectious dose through very close contact between an infected camel and a human being is required for initiation of human MERS-CoV infection by camels. Younger camels may play a particular role in zoonotic transmission since they seem to be more frequently infected and seem to shed more virus than older ones [14-16]. Although the respiratory route is, in our opinion, the most likely route of transmission, a recent paper demonstrated that MERS-CoV survived in raw camel milk slightly longer than in milk of other species [18], suggesting further investigations on a possible food-borne route of transmission. Also, it is not clear yet whether the course of MERS-CoV infection in camels is generally asymptomatic or associated with mild respiratory symptoms, as suggested recently [16].

Conclusions

Phylogenetic analysis and high MERS-CoV viral load in nasal swabs of dromedary camels suggest local zoonotic transmission through the respiratory route.

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Conflict of interest

None declared.

Authors' contributions

NN designed, coordinated and supervised the study and wrote the manuscript; JK performed laboratory testing, analysed data, read and revised manuscript.

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