Evidence for Camel-to-Human Transmission of MERS Coronavirus

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SUMMARY

We describe the isolation and sequencing of Middle East respiratory syndrome coronavirus (MERS-CoV) obtained from a dromedary camel and from a patient who died of laboratory-confirmed MERS-CoV infection after close contact with camels that had rhinorrhea. Nasal swabs collected from the patient and from one of his nine camels were positive for MERS-CoV RNA. In addition, MERS-CoV was isolated from the patient and the camel. The full genome sequences of the two isolates were identical. Serologic data indicated that MERS-CoV was circulating in the camels but not in the patient before the human infection occurred. These data suggest that this fatal case of human MERS-CoV infection was transmitted through close contact with an infected camel.

MERS-COV IS A NEWLY IDENTIFIED HUMAN CORONAVIRUS THAT HAS RE-cently emerged in the Middle East region.[1-3] Current epidemiologic data suggest multiple zoonotic transmissions from an animal reservoir leading to human infection, sometimes with secondary transmission events in humans.[4] In this study, we describe reverse-transcriptase–polymerase-chain-reaction (RT-PCR) detection, isolation, and sequencing of MERS-CoV from a camel and from a patient who died of laboratory-confirmed MERS-CoV infection in Jeddah, Saudi Arabia.

CASE REPORT

A 43-year-old previously healthy Saudi man who had retired from the military was admitted to the intensive care unit at King Abdulaziz University Hospital, Jeddah, on November 3, 2013, with severe shortness of breath. Eight days before admission, fever, rhinorrhea, cough, and malaise developed, followed 5 days later by shortness of breath that gradually worsened. The patient owned a herd of nine camels that he kept in a barn about 75 km south of Jeddah. The patient and three of his friends had been visiting the camels daily until 3 days before his admission. The patient's friends reported that four of the animals (Camels B, F, G, and I) had been ill with nasal discharge during the week before the onset of the patient's illness (Table 1). As reported by his friends, the patient had applied a topical medicine in the nose of one of the ill camels (Camel B) 7 days before the patient's onset of illness. None of the patient's friends had had direct contact with the camels' secretions or mucous membranes. They all remained well during the 60 days that followed the onset of illness in the patient.

Five days after the patient's hospitalization, symptoms of upper respiratory tract

Table 1. Identification of MERS-CoV RNA in Nasal Swabs and MERS-CoV Antibodies in Human and Dromedary Samples.*

Host	Age	Real-Time RT-PCR				IFA	
		Original Nasal Samples†			Culture Isolates‡	Serum Sample 1§	Serum Sample 2¶
		upE	ORF1a	ORF1b			
	yr		*cycle threshold*				
Patient	43	27.5	34.7	33.3	13.2	<1:10	1:1,280
Camel A	9	ND	ND	ND	ND	1:51,200	1:51,200
Camel B‖	<1	36.3	36.9	37.5	11.1	1:160	1:1,280
Camel C	10	ND	ND	ND	ND	1:12,800	1:12,800
Camel D	12	ND	ND	ND	ND	1:1,280	1:1,280
Camel E	12	ND	ND	ND	ND	1:640	1:12,800
Camel F‖	<1	ND	ND	ND	ND	1:80	1:1,280
Camel G‖	<1	ND	ND	ND	ND	1:320	1:640
Camel H	12	ND	ND	ND	ND	1:10	1:640
Camel I‖ **	2	ND	ND	ND	ND	1:10	1:1,280

* IFA denotes immunofluorescence assay, MERS-CoV Middle East respiratory syndrome coronavirus, and ND not detected.
† Results of a real-time reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay are shown for the first samples collected from the patient and the camels. Eluted RNA was screened for the MERS-CoV upstream region of the E gene (upE region) and confirmed by targeting the open reading frame ORF1a and ORF1b regions. Samples with a cycle threshold below 40 were considered to be positive.
‡ The culture isolates tested for the upE region were procured from Vero cells inoculated with the first nasal samples collected from the patient and the camels.
§ Serum sample 1 was obtained on hospital admission from the patient and 5 days later from the camels.
¶ Serum sample 2 was collected on hospital day 14 from the patient and 28 days after the collection of sample 1 (i.e., 33 days after the patient's hospital admission) from the camels.
‖ The presence of rhinorrhea was reported during the week preceding the onset of the patient's illness.
** The first nasal sample from this camel was collected 33 days after the patient's hospital admission.

infection developed in his 18-year-old daughter and resolved spontaneously within 3 days without any complications. After admission, the patient's condition continued to deteriorate, and he died on November 18, 2013. Figure 1 shows a timeline of the main events. Details of the clinical assessment and hospital course are provided in the Supplementary Appendix (available with the full text of this article at NEJM.org), including laboratory investigations (Table S1), chest radiography (Fig. S1), and computed tomography (Fig. S2).
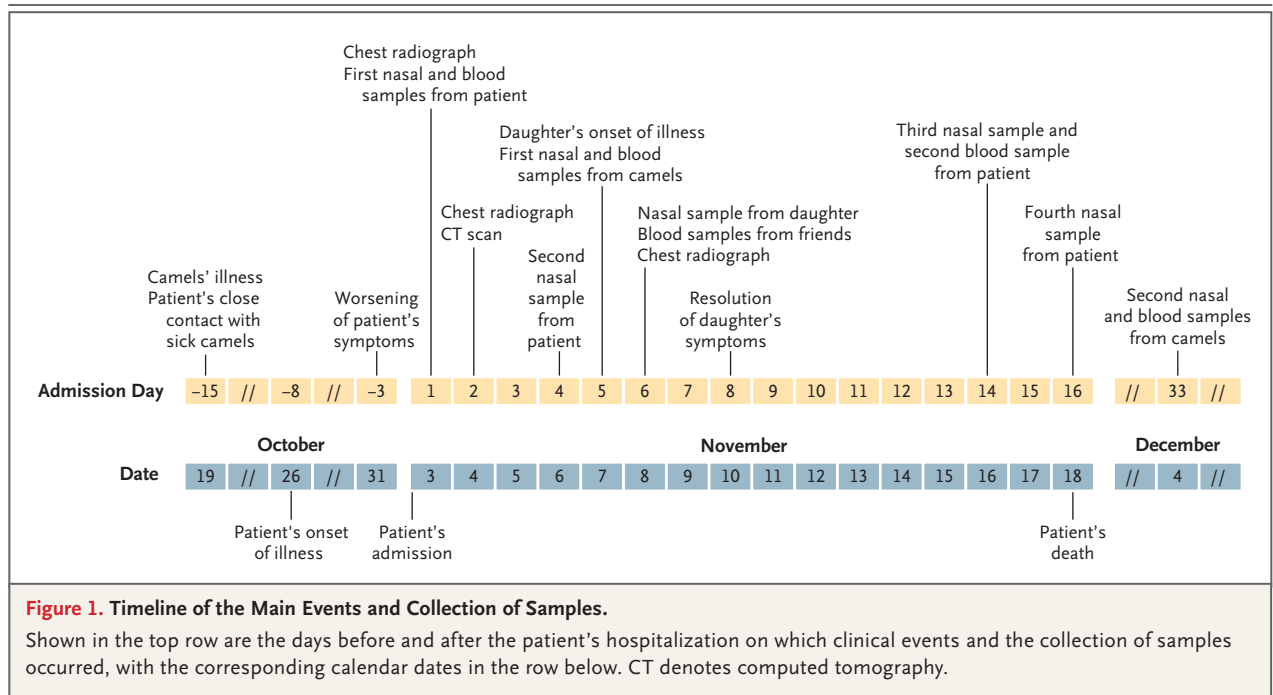
METHODS

FIELD INVESTIGATIONS

Five days after the patient was admitted to the hospital, a veterinarian examined the nine camels, a procedure that was repeated for the following 27 days. During these examinations, the camels were found to be completely healthy, with no nasal discharge.

SAMPLE COLLECTION

Nasal swabs were obtained from the patient on hospital days 1, 4, 14, and 16. Blood samples were also collected from the patient on days 1 and 14. In addition, a nasal swab was obtained from the patient's daughter 1 day after the onset of her illness. Six days after the patient's admission, blood samples were collected from his three friends, who were asymptomatic. Nasal swabs, blood, milk, urine, and rectal swabs were collected from the nine camels 5 days after the patient's admission. Blood and nasal samples were collected again from the camels 28 days later (i.e., 33 days after the patient's hospital admission). All nasal swabs, which were immersed in viral transport medium, and other samples were transported in a cold container to the Special Infectious Agents Unit, a biosafety level 3 laboratory at King Fahd Medical Research Center, King Abdulaziz University, Jeddah, for analysis. Blood samples were centrifuged and serum samples were collected for analysis.

Figure 1. Timeline of the Main Events and Collection of Samples.

Shown in the top row are the days before and after the patient's hospitalization on which clinical events and the collection of samples occurred, with the corresponding calendar dates in the row below. CT denotes computed tomography.

CELL CULTURE

Vero cells (ATCC CCL-81) were inoculated with 100 μl of the nasal swab medium and maintained in complete Dulbecco's Modified Eagle's Medium, as described previously.[4] The cells were incubated in a humidified atmosphere at 37°C in 5% carbon dioxide and examined daily for a cytopathic effect. Cell-culture supernatants were collected when a cytopathic effect was observed and were analyzed by means of real-time RT-PCR. The MERS-CoV isolate that was generated from the first culture passage was used for whole viral genome sequencing.

MOLECULAR DETECTION

RNA was extracted from the nasal swabs or culture supernatants with the use of the QIAamp Viral RNA Mini Kit (Qiagen), according to the manufacturer's instructions. Eluted RNA was screened for the MERS-CoV upstream region of the E gene (upE region) and confirmed by targeting the open reading frame region ORF1a and ORF1b with the use of real-time RT-PCR, as described previously.[2] Further confirmation was performed by partially sequencing the RNA-dependent RNA polymerase (RdRp) and nucleocapsid (N) regions of the viral genome, as recommended by the World Health Organization.[5]

Both assays were conducted on the original samples collected from the patient and from Camel B. (Details regarding the RT-PCR assay and sequencing are provided in the Supplementary Appendix.)

VIRAL GENOME SEQUENCING

Viral RNA extracted from culture supernatants that had been inoculated with samples from the patient and from Camel B were subjected to RT-PCR amplification with an ABI Veriti thermal cycler (Applied Biosystems) with the use of primer pairs covering the whole length of the viral genome. The RT-PCR fragments were then sequenced, as described in the Supplementary Appendix. Sequences were deposited in GenBank and given accession numbers (KF958702 MERS-CoV-Jeddah-human-1 for the patient's isolate and KF917527 MERS-CoV-Jeddah-camel-1 for the camel's isolate). Genomic regions containing unique mutations were also partially resequenced from the original samples obtained from both the patient and the camel.

PHYLOGENETIC ANALYSIS

Sequences were aligned with all MERS-CoV reference strains retrieved from GenBank. Phylogenetic analysis and distance calculations were performed with the use of Molecular Evolution-

ary Genetics Analysis (MEGA) software, version 5, by means of the neighbor-joining method with 1000 bootstrap replicates.

CONVENTIONAL IMMUNOFLUORESCENCE ASSAY

Serum samples that were collected from the patient and the camels were serially diluted up to 1:51,200. A conventional immunofluorescence assay to detect anti–MERS-CoV antibodies was performed in Vero cells infected with MERS-CoV, with the use of a commercially available test kit (Anti-MERS-CoV IFT, EUROIMMUN), as described previously.[2]

RESULTS

Nasal swabs that were collected from the patient on hospital days 1, 4, 14, and 16 were all positive for MERS-CoV upE, ORF1a, and ORF1b regions on real-time RT-PCR (Table 1). The first nasal sample collected from one camel (Camel B) was also positive for the three regions (Table 1). The second sample collected from this camel 28 days later was negative. Nasal samples that were collected from the other camels on day 1 (seven camels) and on day 28 (eight camels) were negative for MERS-CoV RNA (Table 1). The milk, urine, and rectal samples collected from all camels were negative for MERS-CoV RNA. The nasal sample collected from the patient's daughter, who had symptoms of upper respiratory tract infection, was negative for MERS-CoV and H1N1 influenza virus RNA.

Vero cells that had been inoculated with the first samples obtained from the patient and from Camel B showed a cytopathic effect in the form of detachment of cells 3 days after inoculation. Culture supernatants collected 3 days after inoculation with both samples were positive on real-time RT-PCR for the upE, ORF1a, and ORF1b regions. (Table 1 shows the results for the upE region.)

To further confirm these results and to exclude the possibility of cross-contamination between the cultures for the patient and Camel B, RNA samples that had been extracted from the original nasal swabs obtained from the patient and from Camel B were subjected to partial genome sequencing of 242-bp fragments (nucleotides 15049 to 15290) and 312-bp fragments (nucleotides 29549 to 29860) in the RdRp and N regions of the viral genome, respectively. The presence of

Figure 2 (facing page). Phylogenetic Analysis of the Full Spike-Protein Coding Gene and the Full Genome in Human and Camel Samples.

Shown are the phylogenetic trees that were constructed for the gene encoding the full spike protein (nucleotides 21450 to 25511) (Panel A) and the full genome (Panel B). Trees were generated from nucleotide alignments of sequences from culture isolates obtained from both the patient and Camel B and from the Middle East respiratory syndrome (MERS) coronavirus sequences available in the GenBank database. The sequences of the samples obtained from the patient and Camel B are indicated by red squares. The abbreviation cg denotes complete genome sequence, and pg denotes partial genome sequence.

MERS-CoV–specific sequences in these samples was confirmed. Alignment of these two fragments from the two samples confirmed that they were identical.

Full genome sequencing of culture isolates obtained from the patient and from Camel B showed that the two samples were 100% identical. Alignment of the sequences that were obtained in this study with others reported in GenBank showed unique mutations in 14 nucleotide positions (Table S2 in the Supplementary Appendix). Partial genome sequencing of the regions containing these mutations from the original samples obtained from the patient and from Camel B showed the same mutations except for a T-to-C substitution at position 10154 and a T-to-G transversion at position 25800 (Fig. S3 in the Supplementary Appendix). Apart from these differences, there was complete concordance between the two sets of partial sequences obtained directly from the original samples and those obtained from cultures.

Phylogenetic analysis of the gene encoding the full spike protein (nucleotides 21450 to 25511) and the whole genome indicated that the MERS-CoV isolates obtained from the patient and from Camel B were closely related to the Riyadh 3/2013 isolate (KF600613.1), the MERS coronavirus isolate (known as the Munich/Abu Dhabi isolate) (KF192507.1), the betacoronavirus England 1 isolate (KC164505.2), and the human betacoronavirus 2c England-Qatar/2012 isolate (KC667074.1), with 99.8% similarity in sequence identity matrix (Fig. 2). Similar topology was also observed for other viral genes obtained in this study.

The serum sample collected from the patient

A Full Spike Gene
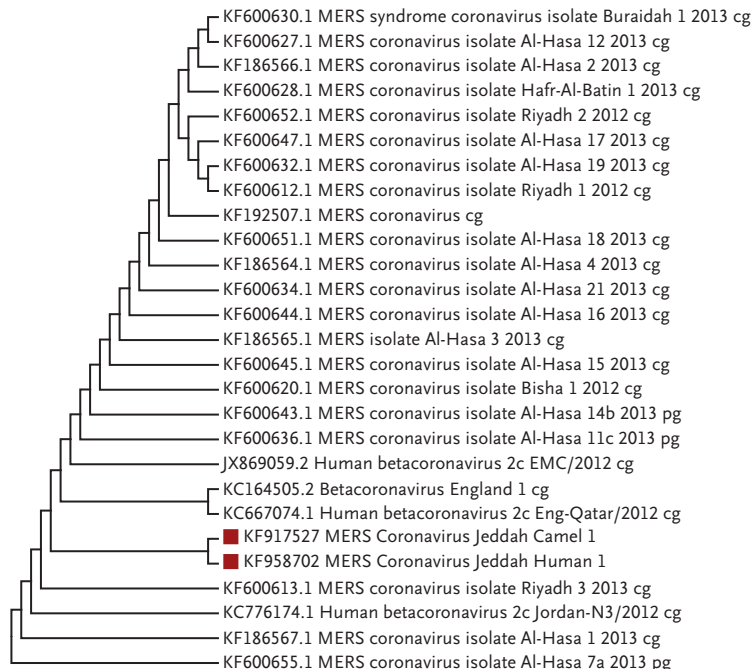
B Full Genome
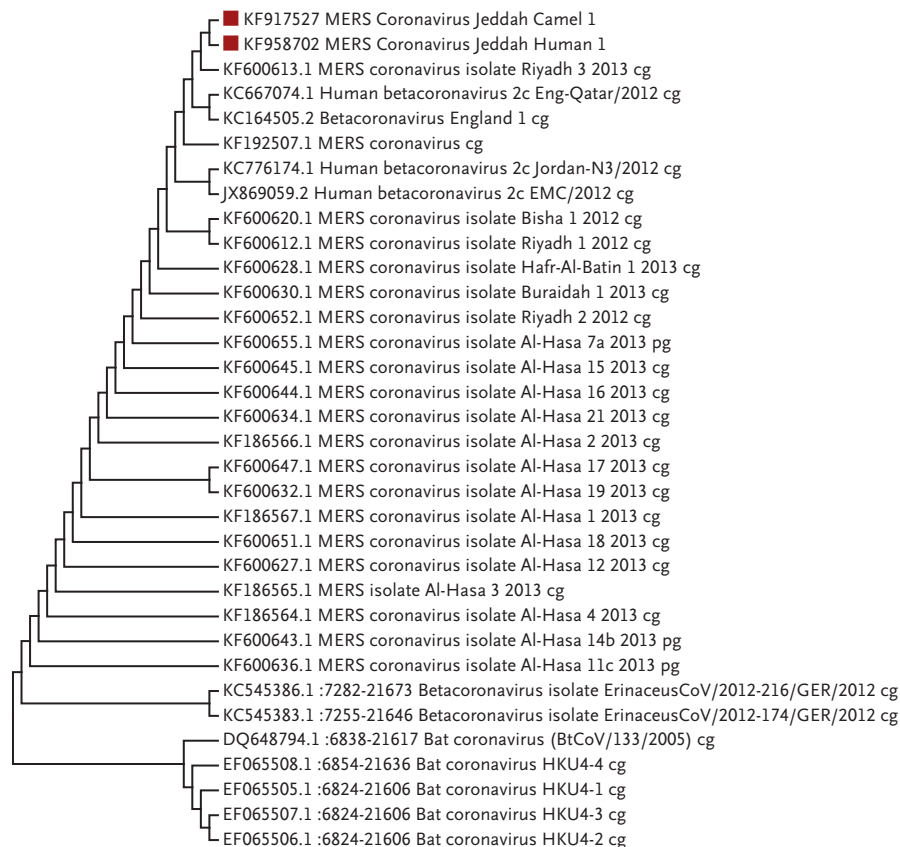
on day 1 was negative for MERS-CoV antibodies (<1:10) on immunofluorescence assay, whereas the sample collected on day 14 showed quadrupling of the antibody titer (1:1280) (Table 1, and Fig. S4 in the Supplementary Appendix). Paired serum samples that were collected from Camel B also showed an increase in the antibody titer by a factor of more than 4, an increase that also occurred in four other camels (Camels E, F, H, and I) (Table 1). The remaining four camels (Camels A, C, D, and G) had high antibody titers in both samples (Table 1). No MERS-CoV antibodies were detected in serum samples obtained from the patient's three friends.

DISCUSSION

The epidemiologic features of the MERS-CoV infections suggest zoonotic transmission from an animal reservoir to humans, perhaps through an intermediate animal host.[4,6-9] Limited secondary transmission from human to human has also been confirmed in both health care and household settings.[6,7,10] Analysis of short genomic sequences indicated that MERS-CoV might have an ancestor in bats.[8,11,12] This hypothesis was further supported by the detection of a small fragment of genomic sequence identical to that of EMC/2012 MERS-CoV Essen isolate (KC875821) in an Egyptian tomb bat (*Taphozous perforatus*) that had been captured in Saudi Arabia.[13] In addition, serologic studies have shown the presence of cross-reactive antibodies to MERS-CoV in dromedary camels in Oman, the Canary Islands, and Egypt.[4,14]

However, the mere presence of antibodies that are cross-reactive to MERS-CoV without simultaneous isolation or PCR detection of the virus from camels in these studies could not confirm their role as reservoirs or intermediate hosts for transmitting the virus to humans. In a recent study, the presence of MERS-CoV RNA was confirmed on real-time RT-PCR assay and partial genome sequencing of the viral RNA in 3 of 14 nasal samples collected from 14 camels on a farm in Qatar and in 2 nasal swabs collected from two patients on the same farm.[15] However, conclusive evidence of transmission from camels to humans or vice versa could not be established.[15]

In our study, the evidence suggests that a dromedary camel was the source of MERS-CoV that infected a patient who had had close contact with the camel's nasal secretions. The presence of identical sequences in the two MERS-CoV isolates recovered from the patient and from Camel B suggests that direct cross-species transmission had probably occurred between the two without any intermediate host. Although cross-contamination between the two samples could be a concern, it is unlikely, owing to the independent times and locations of collection and processing of the two samples. We concluded that the camels had been infected before the patient, since the first serum samples collected from four of the nine camels showed high MERS-CoV antibody titers that did not subsequently increase, as compared with negative MERS-CoV antibody (<1:10) in the patient's first serum sample and the subsequent quadrupling (1:1280) in the patient's second sample, collected 2 weeks later. In addition, active transmission of MERS-CoV among the nine dromedary camels was suggested by a rising MERS-CoV antibody titer and the detection of MERS-CoV RNA and viral isolation from the nasal secretions of Camel B, along with evidence of MERS-CoV antibody titers in the other eight camels that were either high (in four camels) or rising (in four camels). Although it is possible that camels shed the virus intermittently, the clearance of MERS-CoV from the nasal secretions of Camel B and the absence of any detectable MERS-CoV RNA from the secretions of the other camels, despite the serologic evidence of recent infection, suggest that these camels were transiently infected and did not shed the virus subsequently. These two isolates showed close similarity (99.8%) to the Munich/Abu Dhabi isolate (KF192507.1), betacoronavirus England 1 isolate (KC164505.2), human betacoronavirus 2c England-Qatar/2012 isolate (KC667074.1), and Riyadh 3/2013 isolate (KF600613.1). However, unique mutations were identified in the sequences that differentiated them from other, previously reported sequences (Table S2 in the Supplementary Appendix). The nucleotide differences between sequences of the original samples and those of the culture isolates at positions 10154 and 25800 were probably caused by cell-culture adaptive mutations.

In conclusion, these data suggest that MERS-CoV causes a zoonotic infection that can infect dromedary camels and can be transmitted from them to humans through close contact. These data also suggest that the camels were transiently infected, since the virus seemed to be

cleared after the acute infection. Camels may act as intermediate hosts that transmit the virus from its reservoir to humans. The exact reservoir that maintains the virus in its ecologic niche has yet to be identified.

Editor's note: The patient and camels discussed in this article are also described in Memish ZA, Cotten M, Meyer B, et al. Human infection with MERS coronavirus after exposure to infected camels, Saudi Arabia, 2013. Emerg Infect Dis 2014;20:1012-5.

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