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# Asymptomatic MERS-CoV Infection in Humans Possibly Linked to Infected Dromedaries Imported from Oman to United Arab Emirates, May 2015

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In May 2015 in United Arab Emirates, asymptomatic Middle East respiratory syndrome coronavirus infection was identified through active case finding in 2 men with exposure to infected dromedaries. Epidemiologic and virologic findings suggested zoonotic transmission. Genetic sequences for viruses from the men and camels were similar to those for viruses recently detected in other countries.

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Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV) was first detected in humans in 2012 (1). Before 2015, most human infections occurred on the Arabian Peninsula. However, the recent occurrence of MERS in South Korea indicates that this pathogen can cause major outbreaks in other regions (2). Dromedaries are believed to be a source of MERS-CoV (3,4), but only a few case reports provide virologic and epidemiologic evidence that directly supports zoonotic transmission of the virus from dromedaries to humans (5–7). We report the detection of epidemiologically linked MERS-CoV infection in 2 men who had direct contact with infected dromedaries (8,9).

## The Study

A 29-year-old man (contact 1) transported 8 dromedaries from Oman to United Arab Emirates on May 7, 2015 (Table 1). The same day, as part of a national policy for controlling MERS, samples were collected from the dromedaries at a screening center located at the United Arab Emirates border. The samples were tested by reverse transcription

PCR (RT-PCR) on May 10 and found to be positive for the MERS-CoV open reading frame (ORF) 1A and upstream of E genes (10). This finding led local public health authorities to conduct active surveillance on humans who had contact with the infected dromedaries.

A sputum sample collected from contact 1 on May 10, 2015, was tested by RT-PCR on May 12 and found to be positive for MERS-CoV; the man was admitted to a hospital the same day. Follow-up respiratory samples obtained on May 13 and 14 were still RT-PCR-positive, but a sample obtained on May 18 was negative. The patient was asymptomatic at hospital admission and throughout his hospital stay (online Technical Appendix, <http://wwwnc.cdc.gov/EID/article/21/12/15-1132-Techapp1.pdf>).

Contact 2 was a 33-year-old man who worked at the screening center mentioned above. He had direct contact with the same group of infected dromedaries during the sampling procedures. A nasal aspirate sample was obtained from the man on May 14, 2015, and found to be RT-PCR positive for MERS-CoV. Contact 2 was hospitalized on May 18. A follow-up sample obtained on May 18 was RT-PCR negative for MERS-CoV. Contact 2 was asymptomatic throughout his hospitalization (online Technical Appendix).

Samples from 32 other persons were also tested by RT-PCR (online Technical Appendix). None tested positive.

After the initial positive test results, the dromedaries were quarantined. Seven days later (May 14), follow-up nasal swab samples from 5 dromedaries were still positive by RT-PCR (Table 2); the animals also had mucopurulent nasal discharge. The animals were tested for the presence of MERS-CoV-specific neutralizing antibodies (11); all were seropositive. Two 4-month-old calves (ADFCA-HKU1 and ADFCA-HKU2) had the highest virus loads by real-time RT-PCR and the lowest neutralizing antibody titers (Table 2). Nasal swab samples from these 2 dromedaries were also MERS-CoV-positive by rapid antigen testing (12), which suggests the calves were still shedding virus 7 days after the first detection of virus. Virus culture was not attempted. On May 25, 2015, the 2 calves were RT-PCR negative for MERS-CoV, and the whole group of camels was released from quarantine.

Respiratory specimens from the 2 infected humans and the 5 dromedaries that were still positive at the second

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**Table 1.** A chronology of major events in a study of asymptomatic MERS-CoV infection in 2 humans after direct contact with infected dromedary camels imported from Oman to United Arab Emirates, May 2015\*

Date, May 2015	Event
7	Contact 1 transported 8 dromedaries from Oman to the United Arab Emirates border. Contact 2 had direct contact with the dromedaries during sampling procedures at the camel screening center at the border. All 8 dromedaries were quarantined until test results were available on May 10.
10	All 8 dromedaries were found to be RT-PCR positive for MERS-CoV and were quarantined in a separate structure located at the same border location. Active surveillance of persons with direct or indirect contact with the infected dromedaries was initiated. A sputum sample was obtained from contact 1; it tested positive for MERS-CoV by RT-PCR on 12 May, 2015.
12	A sample obtained from contact 1 on May 10 tested positive for MERS-CoV by RT-PCR; contact 1 was hospitalized in a negative-pressure room.
13	A follow-up sample was obtained from contact 1, and it tested positive for MERS by RT-PCR.†
14	A follow-up sample was obtained from contact 1, and it tested positive for MERS by RT-PCR. A nasal aspirate sample was obtained from contact 2; it tested positive for MERS by RT-PCR on May 17.† Samples were obtained from the infected dromedaries, and 5 were still MERS-CoV–positive by RT-PCR (Table 2).†
17	A sample obtained from contact 2 on May 14 tested positive for MERS-CoV by RT-PCR.
18	Contact 2 was admitted to a negative-pressure room in the same hospital as contact 1. Follow-up samples were obtained from contacts 1 and 2, and they tested negative for MERS-CoV by RT-PCR.
20	A follow-up sample was obtained from contact 2, and it tested negative for MERS-CoV by RT-PCR.
21	A follow-up sample was obtained from contact 2, and it tested negative for MERS-CoV by RT-PCR.
25	Follow-up samples from the 5 dromedaries tested negative for MERS-CoV by RT-PCR. All dromedaries were released from quarantine.
End of month‡	Contacts 1 and 2 were released uneventfully from the hospital.

\*Contacts 1 and 2, humans who had direct physical contact with infected dromedaries; MERS-CoV, Middle East respiratory syndrome; RT-PCR, reverse transcription PCR.

†Samples subjected to sequencing analyses.

‡Exact date unknown.

sampling were analyzed by dideoxy sequencing as previously described (13); the nucleocapsid gene sequences of all dromedary samples were found to be identical. Samples from dromedaries ADFCA-HKU1–3 were selected for further analysis, and a sequence contig encompassing the 3' end of the ORF1AB gene through the 3' untranslated region of the MERS-CoV genome ( $\approx 8,900$  nt; sequence coverage 4) was obtained from each sample. Contigs from the 3 samples were identical, with the exception of a V221I (GTT→ATT) mutation in the ORF4b protein of the sample from dromedary ADFCA-HKU2. The viral RNA content of the 2 human samples available for analysis was too low to provide long PCR amplicons (cycle threshold 35.5 and 36.9 by upstream of E gene assay). However, partial sequences of MERS-CoV spike (466 nt, contacts 1 and 2), ORF3–4a (273 nt, contact 1), and nucleocapsid (451 nt, contacts 1 and 2) gene regions could be detected from the

samples. All of these sequences were identical to those deduced from the dromedary specimens. Genomic sequences determined from this study were submitted to GenBank (accession nos. KT275306–KT275315).

The 3 sequence contigs obtained from the dromedary samples were phylogenetically closely related to those of viruses detected in humans in the Saudi Arabia, China, and South Korea in 2015 (Figure). All sequences from this cluster, together with the partial ORF3–4a sequence detected in the sample from contact 1, shared 2 cluster-specific mutations, 79S (TCA→TCT) and P86L (CCT→CTT), in the ORF3 protein, suggesting that these viruses may share a common lineage. Apart from the unique V221I mutation, the sequences for viruses from the 3 dromedaries shared a unique ORF4a-Q102E (GAG→CAG) mutation that was not found in any published MERS-CoV genomes. Other than those mutations,

**Table 2.** Demographic data and clinical test results for MERS-CoV–infected dromedary camels imported from Oman to United Arab Emirates, May 2015\*

Camel ID	Age/sex	Purpose of importation	Mucopurulent nasal discharge†	Test results		
				RT-PCR (C <sub>t</sub> )‡	Rapid antigen test§	Serum neutralizing antibody titer¶
ADFC-A-HKU1	4 m/F	Breeding	Moderate	Pos (24.54)	Pos	1:40
ADFC-A-HKU2	4 m/F	Breeding	Moderate	Pos (27.59)	Pos	1:40
ADFC-A-HKU3	4 m/F	Breeding	Moderate	Pos (28.82)	Neg	1:80
ADFC-A-HKU4	7 m/F	Breeding	Moderate	Pos (29.81)	Neg	1:80
ADFC-A-HKU5	10 y/F	Breeding	Mild	Pos (30.05)	Neg	1:160

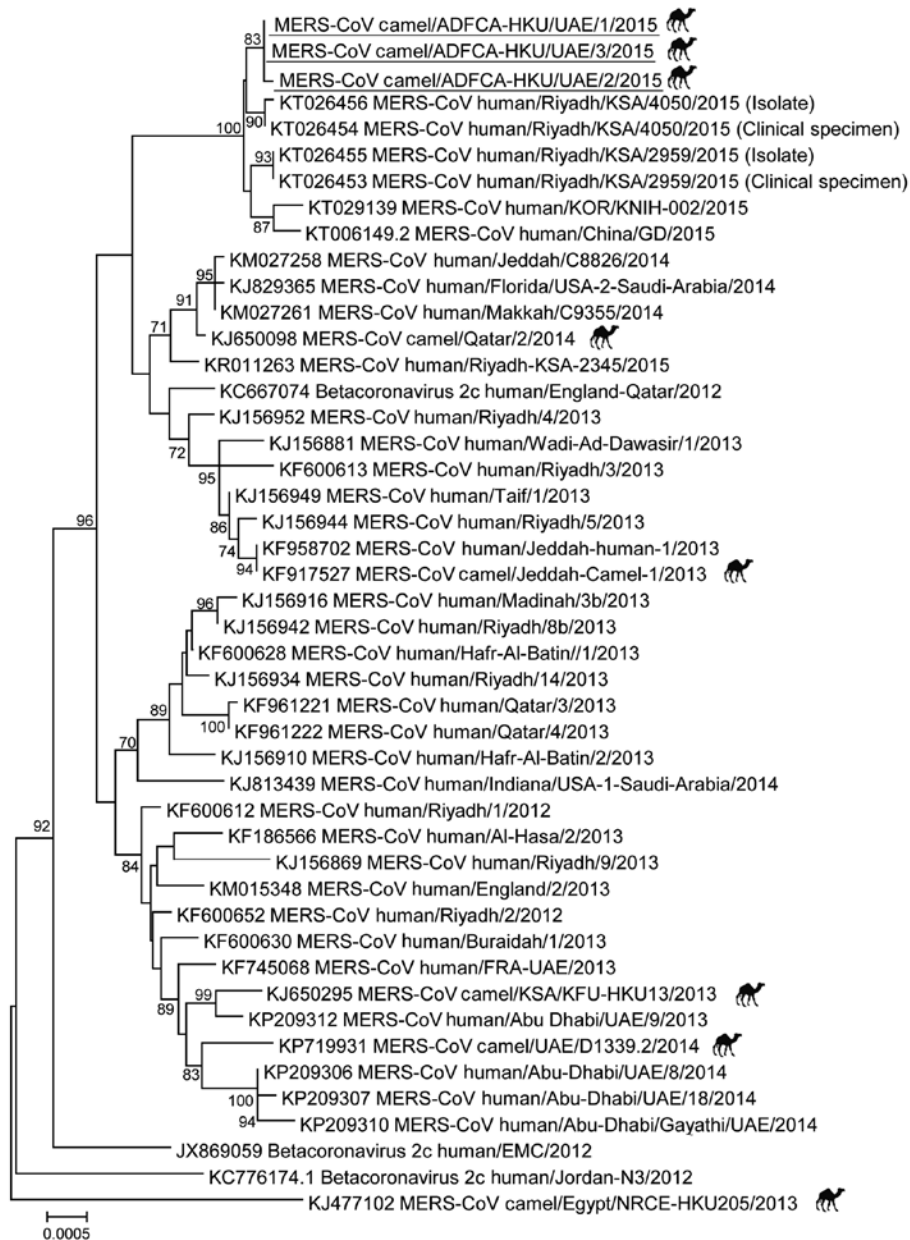
\*C<sub>t</sub>, cycle threshold; ID, identification; MERS-CoV, Middle East respiratory syndrome; Neg, negative; Pos, positive; RT-PCR, reverse transcription PCR.

†Observations or samples from the second sampling on May 14, 2015.

‡Results were determined by open reading frame 1A and upstream of E gene RT-PCR assays; C<sub>t</sub> values were from the open reading frame 1A assay. C<sub>t</sub> values from both assays were comparable (data not shown).

§Results were determined by immunochromatographic tests for MERS-CoV nucleocapsid protein (12).

¶Results were determined by pseudoparticle neutralization assays (11).



**Figure.** Phylogenetic analyses of partial Middle East respiratory syndrome coronavirus (MERS-CoV) genomic sequences for viruses detected in dromedaries imported from Oman to United Arab Emirates, May 2015. A partial viral RNA sequence spanning the 3' end of the open reading frame 1AB gene through the 3' untranslated region of the MERS-CoV genome (~8,900 nt) was used in the analysis. The phylogenetic tree was constructed with MEGA6 software (<http://www.megasoftware.net/>) by using the neighbor-joining method. Numbers at nodes indicate bootstrap values determined by 1,000 replicates. Only bootstrap values >70 are denoted. Underlining indicates sequences for viruses detected in this study. GenBank accession numbers are shown for published sequences. Symbols indicate MERS-CoVs detected from dromedaries. Scale bar indicates the estimated genetic distance of these viruses.

all of the ORFs (nonstructural protein 13, spike, ORF3, ORF5, envelope, membrane, nucleocapsid, and ORF8b) of these virus sequences were unremarkable.

## Conclusions

We report 2 cases of MERS-CoV infection in men who had direct contact with the same group of infected dromedaries. Neither man had a concurrent medical condition or a history of exposure to human MERS cases in the 14 days before their first MERS-CoV-positive test results. Genomic sequences for the viruses derived from the men and dromedaries and findings from the epidemiologic investigation suggest possible zoonotic transmission of MERS-CoV

from dromedaries to humans. Although it is unlikely, we cannot exclude the possibility that the men and dromedaries were independently infected by other sources.

Both infected humans were kept in the hospital for ≈2 incubation periods and were asymptomatic during this period. Clinical observations and positive RT-PCR results suggest that the men were asymptotically infected with MERS-CoV. Asymptomatic infections have been detected previously (14). Our findings provide further evidence that asymptomatic human infections can be caused by zoonotic transmission. It is not clear whether asymptomatic infection can lead to transmission between humans. Nonetheless, our findings highlight the importance of systematic surveillance

of persons who have frequent contact with dromedaries. A recent study demonstrated that persons who have frequent exposure to camels are more likely than the general population to be seropositive for MERS-CoV (4). The unique border screening program and multisectoral collaborations highlighted in this investigation serve as a model for effective MERS-CoV surveillance at the animal–human interface.

Our study had some limitations. We did not test serum samples from the human contacts; such testing would be of interest for follow-up investigation of the patients' serologic responses. We also obtained limited RNA samples from these persons, which prevented us from conducting more extensive viral sequence analyses.

MERS-CoV genomic sequences determined in this study are similar to those of viruses detected in 2015 in patients in Saudi Arabia and South Korea with hospital-acquired infections. The infected dromedaries in this study were imported from Oman, which suggests that viruses from this clade are widely circulating on the Arabian Peninsula. Sequence analyses of MERS-CoVs found in South Korea and China do not suggest that viruses from this clade are necessarily more transmissible variants (15). However, given that a single introduction of MERS-CoV from this clade caused >180 human infections in hospital settings (2) and that viruses of this clade are causing other human infections in Saudi Arabia, further phenotypic risk assessment of this particular MERS-CoV clade should be a priority.

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# Asymptomatic MERS-CoV Infection in Humans Possibly Linked to Infected Camels Imported from Oman to United Arab Emirates, May 2015

## Technical Appendix

### Additional Information<sup>1</sup> Regarding Contacts 1 and 2 and Other Persons Tested in the Study

#### Information Regarding Contact 1

Contact 1 is a truck driver who frequently transports camels from Oman to United Arab Emirates. He confirmed that he had direct contact (i.e. direct physical contact) with the infected camels in this particular transportation. A sputum sample collected on 10 May, 2015, was positive for MERS-CoV by RT-PCR on 12 May, 2015. The man was asymptomatic at the time of sample collection. He was admitted to a negative pressure room in a hospital on 12 May, 2015. At the time of his admission, his temperature was 36.9°C, his heart rate was 78 beats per minute, his blood pressure was 137/54 mmHg, and his respiratory rate was 17 breaths per minute. An aspirate sample was collected on the same day and tested positive for MERS-CoV by PCR on 13 May and he was started on Tamiflu (75 mg bid). The man had no comorbid conditions. He had no history of exposure to MERS patients in the 14 days prior to detection of MERS-CoV. He remained asymptomatic during his hospital stay. He was tested to be negative before leaving the hospital at

the end of May. An official announcement was released by the corresponding health authority (<http://www.haad.ae/haad/tabid/58/ctl/Details/Mid/417/ItemID/487/Default.aspx>).

### **Information Regarding Contact 2**

Contact 2 worked in the border screening center as a cleaner. Because of his job nature, he had frequent direct contact with camels in the center. He was tested twice by RT-PCR assay for MERS-CoV in February 2015 and found to be negative both times. His exposure in May 2015 was considered to be a direct contact (i.e. direct physical contacts). On May 7, 2015, without wearing any personal protective equipment, the man held onto the infected dromedary camels (DCs) while the veterinarian obtained nasal swab samples from the animals..<sup>2</sup>

Nasal aspirate sample taken from Contact 2 on 14 May, 2015, was found to be RT-PCR positive for MERS-CoV. He reported no symptoms at that time. He was admitted to a negative pressure room in a hospital for close monitoring and quarantine. At the time of his admission, his temperature was 36.9°C, his heart rate was 77 beats per minute, his respiratory rate was 16 breaths per minute, and his blood pressure was 132/91 mmHg. The patient did not report any history of comorbid conditions such as cardiovascular diseases, diabetes, chronic pulmonary diseases, rheumatic diseases, liver diseases, renal diseases, or malignant disorders. The patient was interviewed twice for obtaining a detailed history using two different formats. The patient did not report recent history of fever, cough, sore throat, runny nose, earache, chest pain, abdominal pain, body ache, vomiting, diarrhea, headache, or any other complaints. The patient received Tamiflu (75mg bid) starting on May 19, 2015. The patient was not given steroids at that time. The patient did not report any history of recent travel in the 10 days before testing. The patient was interviewed again on May 24, 2015, by an infectious diseases officer using a different form for obtaining additional information related to animal exposure. The patient was interviewed in Arabic. The

patient lived in a compound that is about 10 km from the camel screening center. The patient worked as a cleaner in this center for 20 months (working hours: 72 hours per week). He did not report any history of direct contact with people who had respiratory illness or fever within the 14 days prior to testing. He did not have any recent visits to a hospital. He was tested to be negative before leaving the hospital at the end of May. An official announcement was released by the corresponding health authority

(<http://www.haad.ae/haad/tabid/58/ctl/Details/Mid/417/ItemID/487/Default.aspx>).

### **Information Regarding Additional Persons Screened in the Study**

An additional 32 persons were tested in the study. One of these persons was confirmed to have direct physical contact with the MERS-CoV–positive DCs. The other 31 persons were found to have close contact with the MERS-CoV–positive DCs or humans. None of them was RT-PCR positive for MERS-CoV.

### **Remarks**

<sup>1</sup>Some information from Contacts 1 and 2 was obtained using the ISARIC Case Record Form for Cases of Human Infection with the Novel Coronavirus.

<sup>2</sup>Unlike the practice of Contact 2, full PPE is routinely worn by members in the veterinary team who are in charge of taking camel samples in the screening center. None of the members in the veterinary team had positive results for MERS-CoV since the implementation of the borders screening.