Detection of the Middle East Respiratory Syndrome Coronavirus Genome in an Air Sample Originating from a Camel Barn Owned by an Infected Patient

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ABSTRACT **Middle East respiratory syndrome coronavirus (MERS-CoV) is a novel betacoronavirus that has been circulating in the Arabian Peninsula since 2012 and causing severe respiratory infections in humans. While bats were suggested to be involved in human MERS-CoV infections, a direct link between bats and MERS-CoV is uncertain. On the other hand, serological and virological data suggest dromedary camels as the potential animal reservoirs of MERS-CoV. Recently, we isolated MERS-CoV from a camel and its infected owner and provided evidence for the direct transmission of MERS-CoV from the infected camel to the patient. Here, we extend this work and show that identical MERS-CoV RNA fragments were detected in an air sample collected from the same barn that sheltered the infected camel in our previous study. These data indicate that the virus was circulating in this farm concurrently with its detection in the camel and in the patient, which warrants further investigations for the possible airborne transmission of MERS-CoV.**

IMPORTANCE **This work clearly highlights the importance of continuous surveillance and infection control measures to control the global public threat of MERS-CoV. While current MERS-CoV transmission appears to be limited, we advise minimal contact with camels, especially for immunocompromised individuals, and the use of appropriate health, safety, and infection prevention and control measures when dealing with infected patients. Also, detailed clinical histories of any MERS-CoV cases with epidemiological and laboratory investigations carried out for any animal exposure must be considered to identify any animal source.**

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The Middle East respiratory syndrome coronavirus (MERS-CoV) is a novel human pathogen associated with severe respiratory symptoms and renal failure (1, 2). Since its emergence in 2012, there has been up to 536 laboratory-confirmed infections and 145 deaths in at least 17 countries in Asia, Africa, Europe, and North America (3). Most of these cases originated from countries in or around the Arabian Peninsula, particularly Saudi Arabia (3). The ability of this virus to infect those in close contact with an infected individual, such as family members and health care personnel, as well as the associated high mortality rate may represent a global public health threat (4–7).

Although the source of MERS-CoV and its mode of transmission are not fully understood, zoonotic transmission from an unknown reservoir or through an intermediate host to humans was suggested (7–9). Phylogenetic analysis showed that MERS-CoV belongs to bat-associated clade 2c betacoronaviruses (2, 9, 10). Detection of MERS-CoV-related viruses in Old World insectivorous bats from the family *Vespertilionidae* (11, 12) and the isolation of small genomic fragments identical to the sequence of the Erasmus Medical Center (EMC)/2012 MERS-CoV Essen isolate (GenBank accession number KC875821) from a *Taphozous perforatus* bat in Saudi Arabia (13) suggested that insectivorous bats could be the original source of MERS-CoV. However, due to the limited direct contact between humans and bats and to the detection of neutralizing antibodies to MERS-CoV in dromedary camels from countries like Oman, the United Arab Emirates, Qatar, Egypt, and Saudi Arabia (8, 14–16), dromedary camels were proposed to be involved in the cross-species transmission of MERS-CoV. This was further supported by the detection and partial genome sequencing of MERS-CoV RNA in samples collected from camels in Qatar and Saudi Arabia (17, 18). Anti-MERS-CoV antibodies have also been detected in samples collected from dromedary camels in Saudi Arabia since 1992 (18). While these studies provide a convincing link between humans, camels, and MERS-CoV and indicate that MERS-CoV has been circulating in dromedary camels for a long time, they do not prove that camels passed the virus to humans.

Recently, several MERS-CoV isolates were obtained from nasal

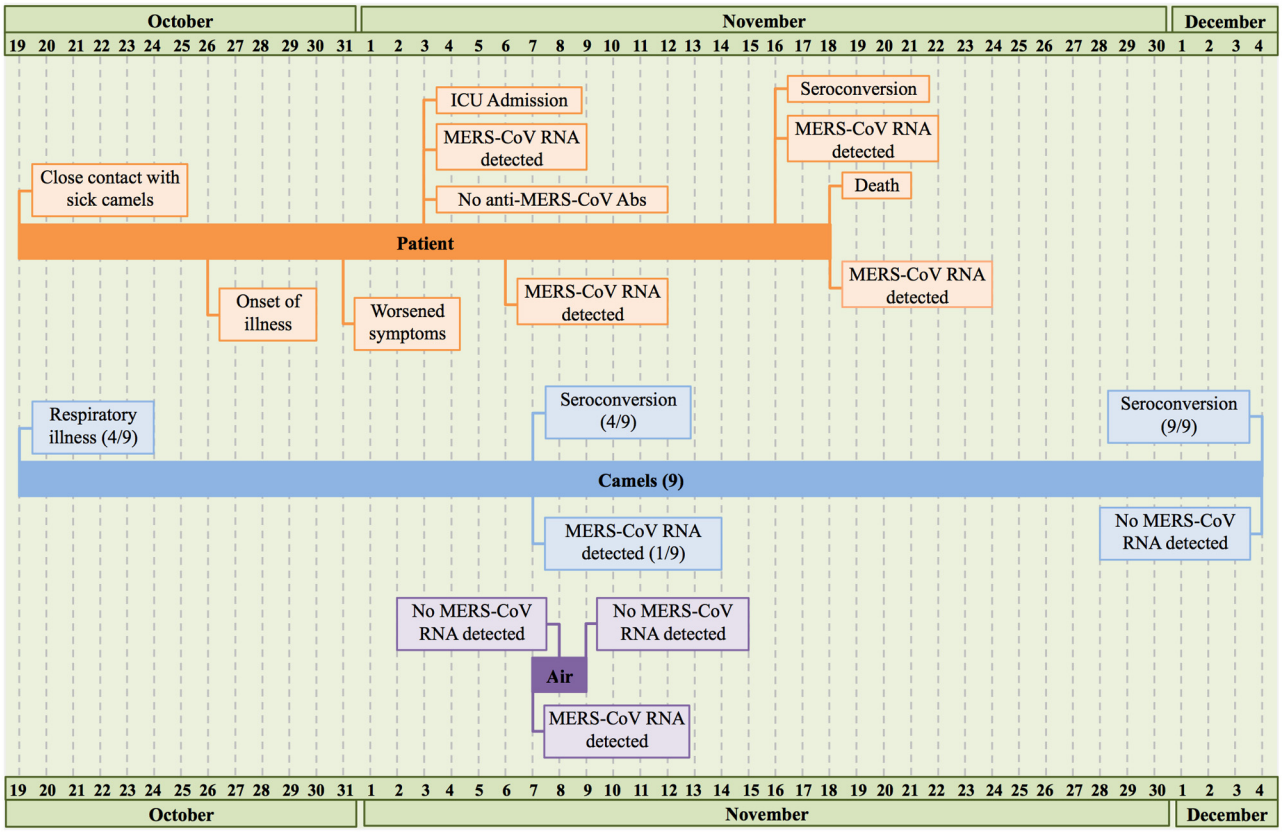

FIG 1 Timeline of the main events in the study. ICU, intensive care unit; Abs, antibodies.

samples from dromedary camels in Saudi Arabia, and their consensus genome sequences were found to be similar to published human MERS-CoV sequences, supporting the role of dromedary camels in human MERS-CoV infections (19). In another report, we also provided evidence for the direct cross-species transmission of MERS-CoV from infected camels to their owner (20). Serological data suggested that the virus was circulating in the herd before infecting the patient. Additionally, we showed based on reverse transcription-PCR (RT-PCR), viral isolation, and full-genome sequencing that both MERS-CoV-Jeddah-human-1 (accession number KF958702) and MERS-CoV-Jeddah-camel-1 (accession number KF917527) isolates were 100% identical and contain characteristic mutations compared to other reported sequences, suggesting direct cross-species transmission from the camels to the patient.

Here, we tried to extend our previous study and to examine whether air could play a role in MERS-CoV transmission. To this end, three air samples were collected from the camels' barn on three consecutive days as shown in Fig. 1. All samples were screened by real-time RT-PCR targeting the upstream region of the E gene (UpE region) of MERS-CoV. Interestingly, only the air sample collected on 7 November 2013, the same day that one of the nine camels in the same barn tested positive for MERS-CoV (Fig. 1), tested positive for the UpE region. The two other air samples were negative for UpE by RT-PCR. Two other confirmatory real-time RT-PCR assays targeting the open reading frame 1a (ORF1a) and ORF1b regions confirmed the UpE-positive result of the first air sample, as shown in Table 1.

To further confirm these results, extracted RNA was subjected to partial genome sequencing of a 665-nucleotide (nt) segment in ORF1a (accession number KJ740999), a 706-nt segment in the RNA-dependent RNA polymerase (RdRp) (accession number KJ741000), a 688-nt segment in ORF1b (accession number KJ741001), a 452-nt segment in UpE (accession number KJ741002), and a 403-nt segment in the nucleocapsid (N) region (accession number KJ741003) of the viral genome. Here, we con-

TABLE 1 Results of a real-time RT-PCR for MERS-CoV RNA in air samples

Day of air sample collection[a]	Threshold cycle of the real-time RT-PCR for detection of indicated MERS-CoV RNA[b]		
	UpE	ORF1a	ORF1b
1	34.7	34.4	33.3
2	ND	ND	ND
3	ND	ND	ND

[a] Samples were collected starting on 7 November 2013.
[b] ND, not detected.

firmed the presence of MERS-CoV-specific sequences in the first air sample and found that these fragments are 100% identical to the corresponding regions in our previous isolates MERS-CoV-Jeddah-human-1 and MERS-CoV-Jeddah-camel-1, obtained from the patient and the infected camel in this barn, respectively (see Fig. S1 in the supplemental material). Of note, further partial genome sequencing of a 697-nt segment of the viral RNA in the ORF1a region (accession number KJ740998) from the air sample showed that the virus is identical to the original isolates obtained from the nasal samples collected from the patient and the infected camel, without the cell culture-adapted mutation (T10154C) observed in our earlier report (see Fig. S2 in the supplemental material).

These data confirm our previous report (20) and show evidence for the presence of the airborne MERS-CoV genome in the same barn that was owned by the patient and housed the infected camels. The detection of viral RNA in the air sample collected on the same day that one of the camels' samples tested positive for MERS-CoV and the fact that all genome sequences obtained from the air sample were identical to those from the camel and the patient samples suggest that the detected viral RNA originated from the camels.

MERS-CoV was reported to be more stable than influenza A H1N1 virus under different environmental conditions on surfaces or in aerosols (21). Specifically, viable MERS-CoV was recovered from surfaces after 48 h at 20°C and 40% relative humidity and after 24 h at 30°C and 30% relative humidity. Similarly, the viability of MERS-CoV decreased by 7% only in aerosols when the virus was incubated at 20°C with 40% relative humidity. However, virus isolation in cell culture was unsuccessful from the air sample collected in the current study, which may be due to a loss of viral infectivity in the collected air sample. Therefore, further studies are clearly needed to confirm the viability of MERS-CoV at different environmental conditions and to confirm its infectivity. Nonetheless, while other routes of transmission, such as droplet contact or fomite transmission, may be involved, the detection of MERS-CoV RNA in the air sample from this barn concurrently with its detection and isolation from the infected camel and the onset of symptoms in the patient warrants further investigations for the possible airborne transmission of MERS-CoV.

The shedding of MERS-CoV into the environment is supported by several reports, including report of the nosocomial infection of immunocompromised patients and the infection of those in close contact with patients, such as family members and health care workers (4–7). Furthermore, the detection of MERS-CoV-neutralizing antibodies and its genome in dromedary camels (8, 14–18) clearly suggest that these animals may play an important role in MERS-CoV transmission to humans. To our knowledge, this is the first report on the possible risk of airborne transmission of MERS-CoV, especially to personnel working directly with infected patients or animals. Our data suggest that camels may be a source of infectious MERS-CoV, which can be transmitted to humans within confined spaces. These results also suggest that air sampling might be a useful approach to investigate the role of the airborne transmission of MERS-CoV spread and shedding. Further studies are urgently needed to fully understand the role of camels in the transmission of MERS-CoV and whether airborne transmission plays a role in MERS-CoV spread in order to implement control and prevention measures to prevent the transmission of this deadly virus.

Air sampling procedure. Air samples were collected from the camels' barn on three consecutive days, with day 1 (7 November 2013) being the same day that one of the nine camels was positive for MERS-CoV by real-time RT-PCR. Samples were collected using the MD8 airscan sampling device (Sartorius) and sterile gelatin filters (80 mm in diameter and 3-μm pore size; type 17528-80-ACD; Sartorius). Air was sampled at a speed of 50 liters/min for 20 min. Filters were dissolved in 5 ml viral transport medium (VTM) and stored at −80°C until analyzed.

Real-time RT-PCR. RNA was extracted from the dissolved filter solution using a QIAamp viral RNA minikit (Qiagen, Germany) according to manufacturer's instructions. Eluted RNA was screened for the UpE region using the real-time RT-PCR assay on a Rotor-Gene Q real-time PCR machine (Qiagen, Germany) as previously described (22). Samples were also tested by real-time RT-PCR for the ORF1a and ORF1b regions for confirmation as described previously (22).

Sequencing and alignment. Further confirmation was performed by partially sequencing the UpE, ORF1a, ORF1b, RdRp, and N regions of the viral genome as per the WHO recommendations (23). In addition, one region containing unique mutations in isolates obtained in our previous report (20) were also sequenced. Sequencing was performed as described previously (20). Sequences were aligned with the genome of the MERS-CoV-Jeddah-camel-1isolate (KF917527) obtained in our pervious study using Geneious 7.0.6 software.

Nucleotide sequence accession numbers. Sequences obtained in this study were deposited in GenBank and given the following accession numbers: MERS-CoV-Jeddah-air-1-2014-ORF1a-partial cds-1, KJ740998; MERS-CoV-Jeddah-air-1-2014-ORF1a-partial cds-2, KJ740999; MERS-CoV-Jeddah-air-1-2014-RdRp-partial, KJ741000; MERS-CoV-Jeddah-air-1-2014-ORF1b-partial, KJ741001; MERS-CoV-Jeddah-air-1-2014-UpE-partial, KJ741002; and MERS-CoV-Jeddah-air-1-2014-N protein-partial, KJ741003.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.01450-14/-/DCSupplemental.

Figure S1, PDF file, 1.3 MB.

Figure S2, PDF file, 0.1 MB.

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