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BRIEF REPORT

Infectious MERS-CoV Isolated From a Mildly Ill Patient, Saudi Arabia

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Middle East respiratory syndrome coronavirus (MERS-CoV) is associated with a wide range of clinical presentations, from asymptomatic or mildly ill to severe respiratory illness including death. We describe isolation of infectious MERS-CoV from the upper respiratory tract of a mildly ill 27-year-old female in Saudi Arabia 15 days after illness onset.

Keywords. asymptomatic; MERS; mild; prolonged detection; virus isolation.

Since its emergence in 2012, Middle East respiratory syndrome coronavirus (MERS-CoV) transmission has been associated with both direct exposure to dromedary camels [1] and with exposure to markedly symptomatic MERS patients, usually in households or health care settings [2–4]. Other potential sources of infection are less clear, but unrecognized transmission associated with infected individuals who are mildly ill or asymptomatic has been suspected [5, 6]. Although MERS-CoV RNA has been detected for prolonged periods from respiratory specimens of confirmed patients who were mildly ill or asymptomatic [7–9], isolation of live MERS-CoV has not been previously documented among this group.

During October 2015, the Saudi Arabia Ministry of Health (MoH) reported a cluster of MERS-CoV infections identified in female janitors working at a university in Riyadh [10, 11]. We summarize below the clinical course and concomitant laboratory investigation of a case identified early in this cluster.

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INITIAL MERS DIAGNOSIS

A 27-year-old expatriate female was tested for MERS-CoV on October 10, 2015, following diagnosis of her roommate with MERS the previous day. She was found to be positive by MERS-CoV-specific real-time reverse transcriptase polymerase chain reaction (rRT-PCR) of a nasopharyngeal (NP) swab and was subsequently admitted to a MERS referral hospital in Riyadh on October 10 for isolation and monitoring. Based on public health investigation at that time, she reported the onset of mild upper respiratory symptoms on September 30, 2015, 10 days before detection and hospitalization (Figure 1); the following findings are presented according to this date, as days post–illness onset.

CLINICAL PRESENTATION AND HOSPITAL COURSE

According to a retrospective review of her medical chart, the patient had rhinorrhea at the time of admission and denied cough, shortness of breath, chest pain, gastrointestinal symptoms, or fever (Figure 1A). She reported no underlying medical conditions. White blood cell counts with differential and blood chemistry analyses were within normal limits throughout hospitalization (Supplementary Table 1). A chest x-ray was obtained 13 days after illness onset (on October 13) and was interpreted as unremarkable. She received oseltamivir, ceftriaxone, and azithromycin empirically beginning at admission. Following her hospital admission, no respiratory symptoms were reported until 22 days after illness onset (on October 22), when the patient developed a mild cough, which was noted for 6 days (Figure 1A); she remained on room air throughout. No hypoxia was identified during hospitalization, and her oral temperature peaked at 37.6°C. Just before discharge, she reported rhinorrhea and was started on oseltamivir following a new diagnosis of influenza. She was discharged 40 days after illness onset (on November 9).

LABORATORY INVESTIGATION

Clinical Testing in Respiratory Specimens

To assess MERS-CoV status, we reviewed hospital records for serial diagnostic testing of respiratory specimens, performed by MoH using rRT-PCR assays targeting MERS-CoV upE and ORF1a genes [12]. The same diagnostic specimens were also routinely tested for influenza A virus (H1N1), and these results were reviewed if available.

Based on hospital records, 16 NP swabs were collected for MERS-CoV diagnostic testing (Figure 1). Twelve NP swabs collected during 10–32 days after illness onset (between October 10 and November 1) were confirmed positive (Figure 1A). Cycle threshold (Ct) values of MERS-CoV upE rRT-PCR were

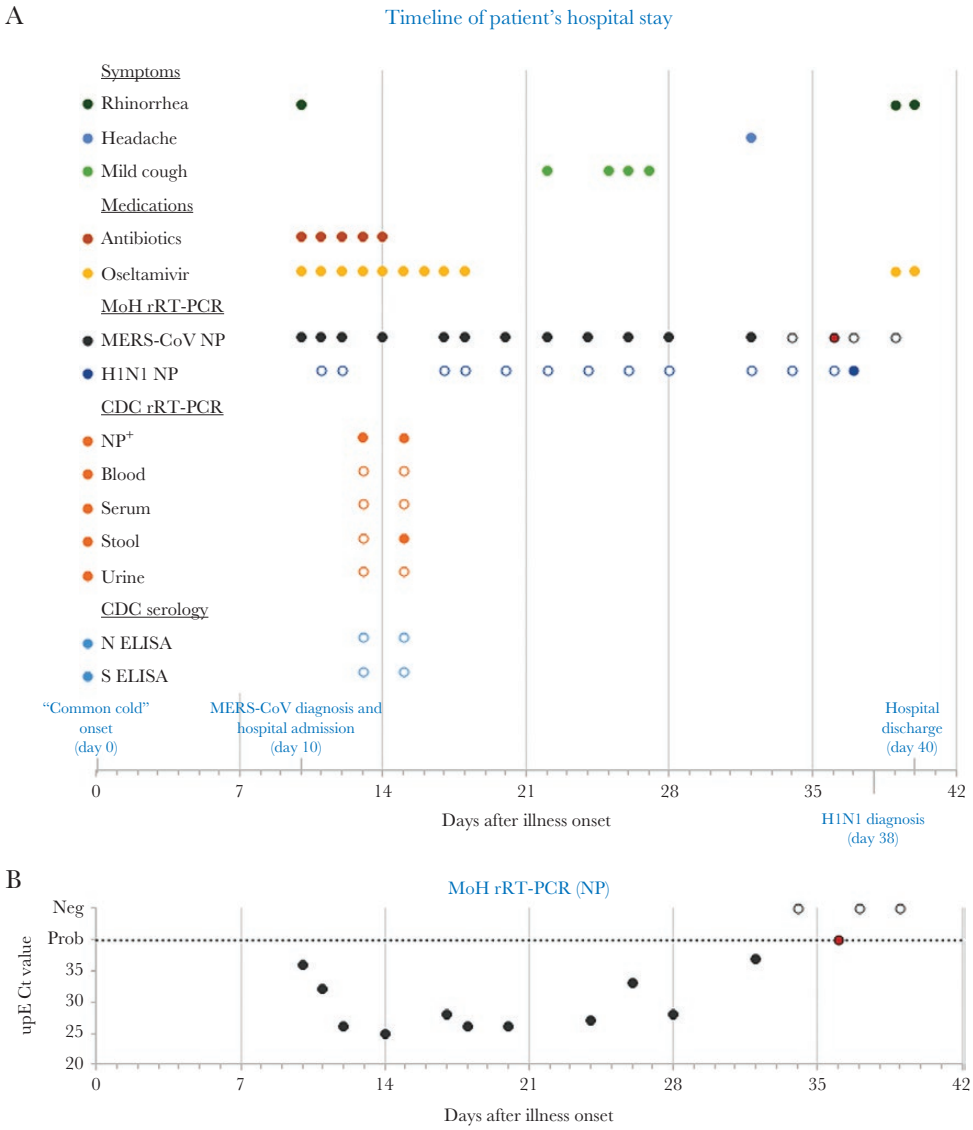

Figure 1. Clinical, laboratory, and hospital findings. A, Timeline of events during the patient's hospital stay, by days after illness onset. Patient-reported illness onset (represented as day 0) was on September 30, 2015. Hospital admission and discharge were on days 10 and 40 after illness onset, respectively. Reported symptoms, treatments, and laboratory tests are depicted. The patient's medical chart was systematically reviewed for administration of medications and for mention of the following symptoms on each day of hospitalization: fever, cough (with description if available), headache, sore throat, dyspnea, chest pain, muscle ache, chills, abdominal pain, vomiting, diarrhea, and other; rhinorrhea was reported under other symptoms. A solid circle means that the medication was administered or the symptom was reported in the chart. Symptoms not depicted were not reported throughout hospitalization. Temperature was also recorded from regular vital sign measurements. We defined fever as a measured oral temperature of >38.0°C or a measured axillary temperature of >37.5°C; she remained afebrile throughout. For test results, a solid circle represents a positive result and an open circle represents a negative result; the red circle at day 36 of the Saudi Arabia Ministry of Health (MoH) real-time reverse transcriptase polymerase chain reaction (rRT-PCR) results represents a probable positive result (only 1 of 2 rRT-PCR assays was positive; cycle threshold (Ct) values not available). Nasopharyngeal (NP^+): live virus was also isolated from the 2 NP specimens submitted to the Centers for Disease Control and Prevention (CDC). B, Middle East respiratory syndrome coronavirus (MERS-CoV) upE rRT-PCR Ct values obtained from MoH diagnostic testing by days after illness onset. The dashed line represents the limit of detection, above which specimens were considered MERS-CoV-negative (open circle). Probable positive (red circle) results were assigned a value of 40 for graphing purposes. A Ct value was not available for the specimen collected 22 days after illness. Abbreviations: ELISA, enzyme-linked immunosorbent assay; N, nucleocapisd; S, spike.

available for 11 of the 12 positive specimens and ranged from 25 to 37 (Figure 1B). On day 10 after onset, the upE Ct value was 37, indicating a low viral load; by day 14, the Ct value was much lower, at 25, indicating a notable rise in viral load. An NP swab collected 36 days after illness onset was probable for MERS-CoV, meaning that 1 target was detected but the other was not; target-specific results were not available. The final 2 NP swabs collected 37 and 39 days after illness onset tested negative. The swab collected on day 37 tested positive for influenza A virus (H1N1) by rRT-PCR. Among the 14 NP swabs collected during days 10–37, 12 tested negative for H1N1, and results for the remaining 2 were not available (Figure 1A).

MERS-CoV Detection and Sequencing

For additional molecular and serologic analysis at the US Centers for Disease Control and Prevention (CDC), 2 (of each) NP, whole blood, sera, stool, and urine specimens were collected on days 13 and 15 after illness onset (on October 13 and 15); NP swabs were collected in 2 mL of viral transport medium. All specimens were frozen and shipped on dry ice. At the CDC, stool suspensions were prepared in phosphate-buffered saline (10% weight/volume). RNA was extracted from 200-μL sample aliquots on a NucliSens EasyMAG (BioMerieux), recovering 100 μL of total nucleic acid. Testing was performed by MERS-CoV upE and N2 and/or N3 rRT-PCR assays [13]. All specimen extracts were tested neat; stool extracts were also diluted 1:5 in nuclease-free water to remove potential stool inhibitors. Genome sequencing was attempted on positive specimens with Ct values ≤36, as described previously [14]. Nucleotide sequences were aligned using Clustal X, version 1.83, implemented in BioEdit, version 7.2.5. Phylogenetic analyses were performed using MrBayes 3.2.6 under a GTR model of nucleotide substitution with 4 categories of γ-distributed rate heterogeneity and a proportion of invariant sites (GTR + 4 + I).

MERS-CoV RNA was detected in the 2 NP swabs, and 1 stool specimen (at 1:5 dilution) was collected 15 days after illness onset. Estimated viral loads, based on the upE assay, were 4.8×10^6 and 9.6×10^5 genome copies/mL in the NP specimens collected on days 13 and 15, respectively, and 1.0×10^4 genome copies/gm of stool from day 15. Whole-genome sequences were obtained from the 2 sequential NP swabs and were 100% identical (accession numbers: MG520075-MG520076). They showed 99.9% similarity and closest phylogenetic clustering with sequences from 4 severely ill MERS patients who were linked to a hospital outbreak in Riyadh in August 2015 (accession numbers: KU851860-851862 and KU851864) [15].

MERS-CoV Isolation

The 2 MERS-CoV RNA-positive NP specimens submitted to the CDC (collected on days 13 and 15) were serially diluted 10-fold in DMEM in a 96-well plate, and subsequently used to inoculate Vero cell suspensions. The cells were observed daily between days 3 and 7 postinoculation; cytopathic effect (CPE) was observed under inverted scope 3 days postinoculation. Any wells that exhibited CPE were harvested and passaged in a 24-well plate. RNA was extracted from 50 μL of the potential virus lysate. Both lysates were confirmed positive for MERS-CoV by N2 rRT-PCR assay; Ct values were 12 (day 13) and 15 (day 15). The isolated viruses (accession numbers: MG546330-MG546331) were subsequently sequenced using Fluidigm Access Array PCR and MiSeq amplicon sequencing [16]. Whole-genome sequences of the 2 isolated viruses were identical to their clinical specimens, except the day 13 isolated virus, which had mixed bases at positions 23 364 and 25 861 (nucleotide location based on accession number JX869059.2). The variant alleles of the isolate would predict amino acid changes (N>D in spike [S] protein and V>L in ORF4a) that may reflect adaptation in culture. Virus isolation was not attempted for the MERS-CoV RNA-positive stool specimen because of low virus load.

Antibody Responses

Sera collected at days 13 and 15 after onset were examined for the presence of MERS-CoV-specific antibodies against the nucleocapisd (N) and S proteins, as previously described [17]. Both specimens were below the limit of detection for both N and S enzyme-linked immunosorbent assays (ELISAs). No sera were available after day 15.

DISCUSSION

We describe isolation of live MERS-CoV from the upper respiratory tract of a mildly ill patient in Saudi Arabia. The ability of a mildly ill MERS patient to shed live virus has not previously been documented and fills an important gap in our understanding of MERS-CoV natural history.

The patient's illness began with reports of upper respiratory tract symptoms, or "a common cold." Rhinorrhea was noted 10 days later, when she was admitted to the hospital for isolation, after which no symptoms were reported for a further 12 days. A mild cough then began 22 days after illness onset and lasted for 6 days. Despite this mild cough, she was noted to be without hypoxia, dyspnea, chest pain, or other signs/symptoms of respiratory illness throughout hospitalization. MERS-CoV RNA was detected in her stool specimen (at day 15) and up to 32 days after illness onset in upper respiratory specimens; low virus load in the stool was coincident with the highest virus loads measured in the upper respiratory tract and may reflect shedding from that compartment. Most notably, live virus was isolated from the NP swabs collected 13 and 15 days after illness onset, when she was reported to be asymptomatic. N and S ELISA antibody responses were not detected by day 15 of illness. We were unable to test subsequent serum specimens.

The patient was admitted to the hospital for isolation following MERS-CoV detection. Home isolation may be considered for asymptomatic or mildly ill patients who test positive for MERS-CoV [18], but this case patient resided in shared accommodation, and home isolation was not practical. Although the role of mildly ill patients in transmission is not fully understood [19], the ability of these patients to shed infectious MERS-CoV, as demonstrated here, should inform home isolation considerations.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Disclosure. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Ethical approval. The patient provided informed written consent. The study was reviewed and approved by the Saudi Arabia Ministry of Health Institutional Review Board before initiation.

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