

Factors Associated With Viral Load Kinetics of Middle East Respiratory Syndrome Coronavirus During the 2015 Outbreak in South Korea

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We conducted a retrospective study of Middle East respiratory syndrome coronavirus (MERS-CoV) viral load kinetics using data from patients hospitalized with MERS-CoV infection between 19 May and 20 August 2015. Viral load trajectories were considered over the hospitalization period using 1714 viral load results measured in serial respiratory specimens of 185 patients. The viral load levels were significantly higher among nonsurvivors than among survivors ($P = .003$). Healthcare workers ($P = .001$) and nonspreaders ($P < .001$) had significantly lower viral loads. Viral RNA was present on the day of symptom onset and peaked 4–10 days after symptom onset.

Keywords. Middle East respiratory syndrome coronavirus; viral load; virus shedding; healthcare-associated infections; infectious disease transmission.

Between April 2012, when the Middle East respiratory syndrome (MERS) was identified, and the end of November 2019, a total of 2494 laboratory-confirmed cases were reported across 27 countries, with a case fatality rate of 34.4% [1]. Between May and July 2015, the largest outbreak of MERS outside the Middle East occurred in South Korea, originating from a single imported case, resulting in 186 individuals with laboratory-confirmed infection and 38 deaths [2, 3].

In humans, infection with MERS coronavirus (MERS-CoV) often results in severe illness and death, particularly in older individuals with underlying disease [4]. The viral load level and disease severity have been reported to be correlated [5–8]. However, data on epidemiological factors related to viral load kinetics are limited.

Central epidemiological characteristics of the MERS outbreak in South Korea included super-spreaders and nosocomial infections [2, 3]. Super-spreaders transmitted the virus to a large number of contacts before isolation, had a significantly longer interval between hospital admission and isolation, and had very low cycle threshold values at diagnosis [9]. In previous studies, specimens were not collected sequentially during the course of illness, and thus the viral load trajectories between spreaders and nonspreaders have not been compared.

We investigated the viral load kinetics and the correlation between disease outcome and other clinical or epidemiological factors to provide scientific evidence for public health prevention and decision-making for future outbreaks.

MATERIALS AND METHODS

This retrospective study analyzed the laboratory records of 185 patients with confirmed MERS in 2015. Age was divided into quartiles. The incubation period (IP) was defined as the interval from the presumed infection time to symptom onset. Emergency room (ER) admission was defined as having been admitted to the ER due to severe illness after MERS-CoV infection, and was regarded as one of the clinical outcomes. Super-spreaders and spreaders were defined as patients with confirmed MERS who were epidemiologically suspected of transmitting MERS-CoV to ≥ 5 and < 5 persons, respectively [9]. Clinical data and specimens were anonymized prior to the analysis.

The institutional review board of the Korea National Institute of Health approved this study (approval number 2016-05-08-P-A).

Laboratory testing was performed according to the World Health Organization guidelines [10]. Laboratory results included cycle threshold (Ct) values for the upstream of the envelope E (upE) gene and open reading frame (ORF) 1a targets, from 15 public diagnostic institutes, 5 private clinical diagnostic centers, and 19 participating hospitals, using 3 commercial kits (PowerChek MERS Real-time PCR, Kogene Biotech, Korea; DiaPlexQ MERS Virus Detection, SolGent, Korea; and AccuPower MERS-CoV Real-Time RT-PCR, Bioneer, Daejeong, Korea) [10]. Raw RNA concentrations were transformed to absolute viral loads by conversion factors for each method.

Sputum samples were tested to confirm MERS-CoV infection. Subsequent viral loads tests were performed irregularly based on clinician demand. Viral RNA extraction was performed on sputum samples pretreated with phosphate-buffered saline to reduce viscosity [9]. The upE region was preferred to ORF1a for all analyses performed using Ct values because the upE region is noncoding and is thus less influenced by the presence of messenger RNA. The Ct values between upE and ORF1a are strongly correlated [7].

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The date of the first symptom was defined according to the national MERS control guidelines issued by the Korean Centers for Disease Control and Prevention (KCDC), and all timed data were calculated from this point. In asymptomatic individuals and those who developed respiratory symptoms before contact with MERS-CoV-infected patients (as the source of infection), the day of the first positive laboratory test was defined as day 0 [8].

A general linear model analysis of variance was used for comparisons of viral load levels in different groups. The post hoc test (Scheffe test) was used for the comparisons for spread and stage of transmission of viral loads. The association between IP, death, and survival was calculated using the Kaplan–Meier method and differences between the groups were determined using the log-rank test. Logarithmic transformation was applied to variables with non-Gaussian distribution. All analyses were performed using SAS version 9.4 (SAS Institute, Cary, North Carolina). Statistical tests were 2 sided, and $P < .05$ was considered to indicate statistical significance.

RESULTS

Between 19 May and 22 November 2015, laboratory results from 185 individuals with confirmed MERS-CoV infection

were reported to the KCDC. A total of 1714 laboratory results of serial respiratory specimens measured at irregular intervals from 19 days prior to symptom onset to 169 days after symptom onset were considered in the analysis. The majority of viral loads were measured 0–69 days after symptom onset. There were 38 deaths due to MERS-CoV, which occurred 2–177 days after symptom onset (mean, 15 days). The time of laboratory diagnosis of MERS-CoV infection on RT-PCR ranged from 0 to 18 days after symptom onset (mean, 5.8 days).

The mean time from symptom onset to virus detection was 17 days from onset, and 90% of patients had virus detected within 29 days of symptom onset (Figure 1A). The median incubation period from exposure to first MERS-CoV detection was 6 days overall, and was significantly shorter among the nonsurvivors (fatal cases) than among the survivors (median, 4.3 days and 6.0 days, respectively; log-rank test $P = .0491$, Figure 1B).

Viral load levels peaked 4–10 days after symptom onset, after which they gradually declined (Figure 1C). Viral load levels were substantially higher among patients who died than among survivors (Figure 2A). Patients who were admitted to the ER had significantly higher viral load levels than other

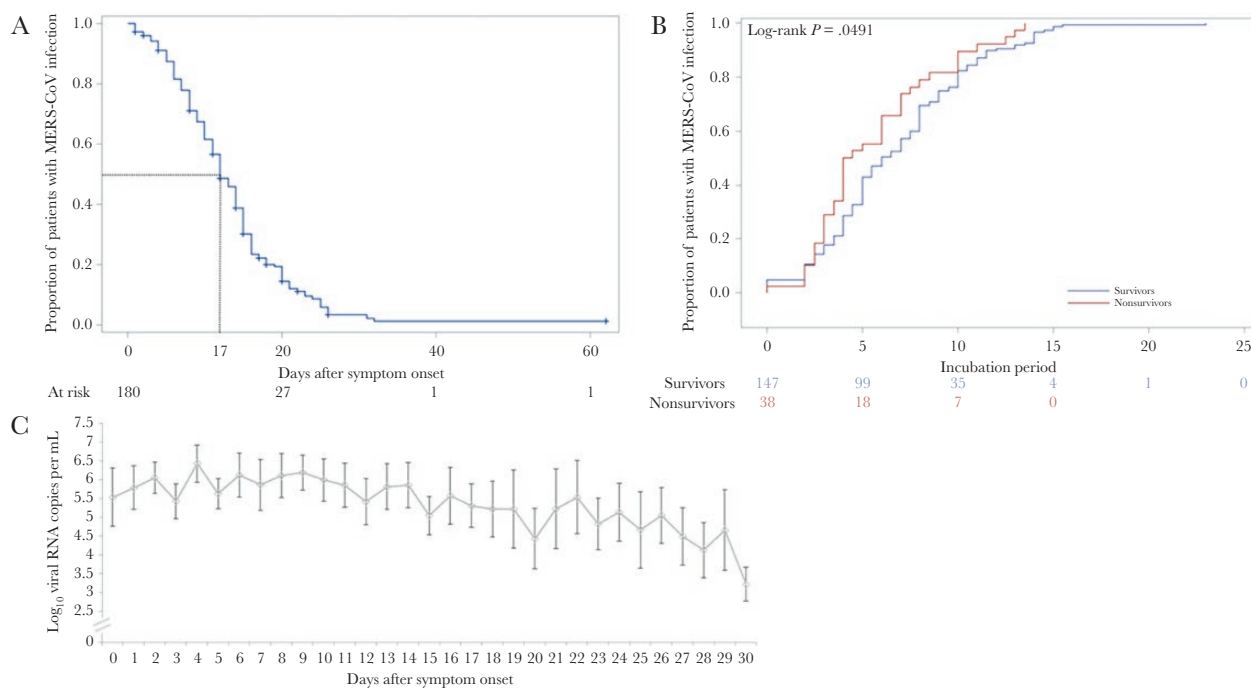


Figure 1. Time to confirmation of Middle East respiratory syndrome coronavirus (MERS-CoV) infection according to the time since symptom onset; incubation period according to the time since exposure; and viral load levels according to the time since symptom onset in 185 patients with laboratory-confirmed MERS-CoV infection. *A*, Kaplan–Meier plot of the time from symptom onset until confirmation of diagnosis of MERS-CoV infection on real-time reverse-transcription polymerase chain reaction (rRT-PCR). *B*, Reverse Kaplan–Meier plot of the incubation period according to survivor status. The x-axis shows the time since exposure to infection, and the y-axis shows the proportion of patients with MERS-CoV detectable on rRT-PCR, stratified by survivor status. The nonsurvivors (fatal cases) had a significantly shorter incubation period than the survivors ($P = .0491$, log-rank test). By day 5 after exposure to infection, 45% of the nonsurvivors and 30% of the survivors had MERS-CoV detectable on rRT-PCR. The median incubation period was 4.3 days in the nonsurvivors and 6.0 days in the survivors. *C*, Mean viral load at 1-day intervals. The error bars show the standard deviation. Viral load levels peaked 2–4 days after symptom onset, after which they gradually declined.

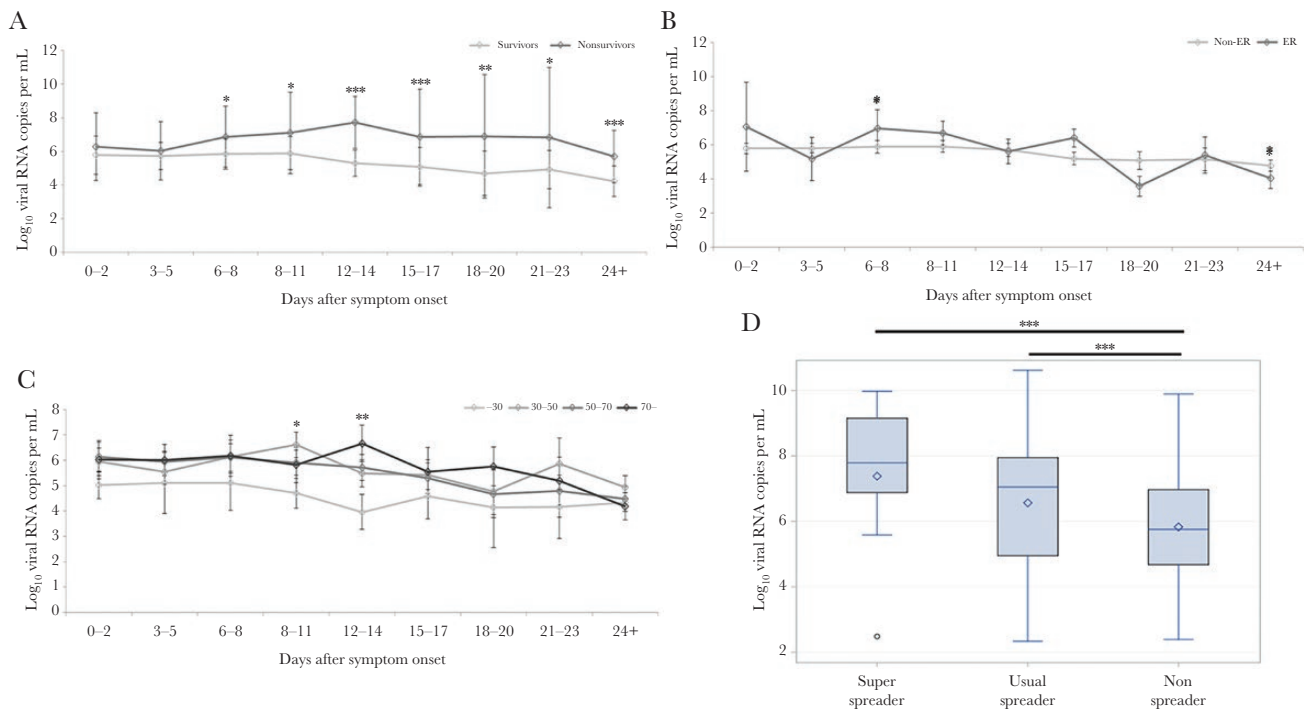


Figure 2. Comparison of viral load levels in 185 patients with laboratory-confirmed Middle East respiratory syndrome coronavirus infection according to the time since symptom onset by vital status (survivors and nonsurvivors) (A), emergency room (ER) admission (B), age (C), and comparison of viral load levels by spreader status (D). In A–C, the points indicate the mean viral load level and the error bars indicate the standard deviation. In D, patients were classified according to the number of contacts who became infected during the 11 days after symptom as super-spreaders (≥ 5 contacts infected, $n = 5$), usual spreaders (< 5 contacts infected, $n = 17$), and nonspreaders (0 contacts infected, $n = 163$). The box-and-whisker plots show the mean (diamond), median (bar) quartiles (top and bottom of the boxes), and range of the viral loads. *P* values were determined using a post hoc (Tukey) test for viral load according to spreader grade, and adjusted by age. **P* < .05; ***P* < .001; ****P* < .001.

patients 6–8 days after symptom onset, but the differences between groups were not marked (Figure 2B). Patients aged >70 years had higher viral loads 12–14 days after symptom onset and patients aged <30 years had lower viral load levels throughout (Figure 2C). Viral load levels were slightly higher among males than among females (Supplementary Figure 1A). Patients with shorter IPs had significantly higher viral loads, especially during the period 15–20 days after symptom onset (Supplementary Figure 1B).

Overall, the 22 spreaders (5 super-spreaders and 17 spreaders) had significantly higher viral load levels than the 163 nonspreaders (Figure 2D), especially during the first 11 days after symptom onset, the main time period during which spreaders transmitted MERS-CoV. The mean viral load level at the time of diagnosis was significantly lower in healthcare workers than in other groups (Supplementary Figure 2).

Among the 185 confirmed cases of MERS-CoV infection, only 3 cases were asymptomatic, all of which occurred among healthcare workers. The 3 individuals with asymptomatic infection had viral RNA, which was detectable at irregular intervals for up to 17 days after the first positive laboratory test. In 1 patient with immunosuppression, viral RNA was still detectable 169 days after symptom onset.

DISCUSSION

Viral load reflects the dynamic interaction between MERS-CoV replication and the ability of the host immune defence response to eliminate the virus. We studied the viral load kinetics of 185 confirmed MERS-CoV cases that occurred during the 2015 outbreak in South Korea. Viral load kinetics was found to be associated with vital status, ER admission, age, sex, IP, spreading events, and case category across different time points and periods of analysis.

Consistent with earlier studies [5–8], this study demonstrated that viral loads were positively correlated with severe clinical outcomes. The viral load level is an important predictor of disease severity and death [5, 6, 11]. A previous analysis revealed that older age and the presence of underlying diseases were associated with a higher mortality rate [4]. As with our study, age was not an independent risk factor for disease severity. Approximately 43.5% of the individuals with MERS in the 2015 outbreak in South Korea were aged >50 years, and the majority of deaths occurred in individuals aged >48 years [3]. Consistent with these findings, the viral loads in our study were significantly higher in patients aged ≥ 50 years than in those aged <50 years.

The reported association between a shorter IP and a higher risk of death may be related to a higher initial infective dose

leading to a faster viral replication [12]. Fatal cases had shorter IPs than those of survivors, although there was no significant difference in viral load per patient group by IP. During the MERS outbreak in South Korea, the majority of the MERS-CoV transmissions occurred within 11 days after symptom onset [9, 13]. Higher viral load level among spreaders during this period was an essential risk factor for spread.

Most MERS cases in Korea had associated nosocomial infections [2, 3]. Higher viral loads were seen in groups with higher exposure such as paid caregivers and family members who had taken care of patients. Healthcare workers have an even higher risk of exposure to MERS-CoV during medical procedures. However, lower viral load levels were found among healthcare workers in the age-adjusted analysis. This is probably attributable to the greater opportunity for early diagnosis and treatment among healthcare workers and their compliance with the wearing of safety equipment and attention to hygiene. The cases that occurred among healthcare workers were asymptomatic to mild. The prolonged detection of MERS-CoV RNA observed in our study is similar to another report [14]. The elevated viral load level in individuals with asymptomatic infection reflects the potential for disease transmission by asymptomatic healthcare workers.

The viral loads of patients with positive MERS-CoV RT-PCR results peaked 2–4 days after symptom onset. The kinetics of the viral RNA load showed a similar but inverse V-shaped pattern, with the highest values 4–10 days after symptom onset and a steady decrease thereafter. The peak period corresponded to the period during which the risk of transmission was highest. A study in Saudi Arabia [6] found that viral loads were highest 3–7 days after diagnosis and decreased gradually until 19 days after diagnosis. Taking into account the approximately 8-day period between infection and diagnosis, these figures suggest that the viral load level is highest 11–15 days after symptom onset [6]. Other Saudi Arabian and Korean studies revealed that the viral load level was higher in lower respiratory tract specimens of patients with severe infection, peaked during the second week of illness, and persisted beyond 21 days after symptom onset; in milder cases, initial MERS-CoV detection, peak, and the end of virus shedding occurred at earlier time points [5, 8].

The earlier time to peak viral load level and longer duration of viral shedding with relatively high viral load found in our study may be due to the participant profile, which included patients with MERS-CoV infections of variable clinical severity [5–8]. Our results showing a viral load peak within 5 days after symptom onset more closely resemble those reported among patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection than those among patients with severe acute respiratory syndrome coronavirus infection. The persistent viral shedding for >4 weeks with a relatively high viral load and slow decline of viral RNA concentration is also similar

to that reported in patients with SARS-CoV-2 infection [15, 16]. The prolonged viral shedding (>169 days) observed in an immunosuppressed patient in this study may have been an indication of poor host immunity.

There are some limitations of this study. It revealed a skewed pattern of viral load kinetics. Viral RNA loads may not accurately reflect molecular viral shedding. Most of the viral loads were measured on sputum samples. It is difficult to standardize the quality of sputum samples. Furthermore, serial analysis for viral load kinetics may also be affected by the absence of specimens on consecutive days. Moreover, detection of viral RNA is not the same as isolation of the infectious live virus, although quantitative detection of MERS-CoV viral RNA is a reasonable proxy measure for determining infectivity [17].

The results of this study provide important information for patient management and outbreak control. Viral shedding can occur for relatively long periods; therefore, strict infection control measures should be implemented during the at-risk period to prevent viral spread in hospitals.

Supplementary Data

Supplementary materials are available at *The Journal of 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.

Notes

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Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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