

Stability of Middle East respiratory syndrome coronavirus (MERS-CoV) under different environmental conditions

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The stability of Middle East respiratory syndrome coronavirus (MERS-CoV) was determined at 20°C – 40% relative humidity (RH); 30°C – 30% RH and 30°C – 80% RH. MERS-CoV was more stable at low temperature/low humidity conditions and could still be recovered after 48 hours. During aerosolisation of MERS-CoV, no decrease in stability was observed at 20°C – 40% RH. These data suggest the potential of MERS-CoV to be transmitted via contact or fomite transmission due to prolonged environmental presence.

In 2012, a novel coronavirus termed Middle East respiratory syndrome coronavirus (MERS-CoV) emerged in the Middle East [1]. Limited human-to-human transmission of MERS-CoV has been observed [2-5] and currently no data are available on the modes of transmission. The occurrence of MERS-CoV as a respiratory pathogen and the high viral loads detected in samples from the lower respiratory tract of infected patients [6,7] suggests that MERS-CoV will be predominantly shed during coughing and via exudates from the lower respiratory tract. For influenza A virus it is shown that transmission is linked to the viability of the virus under different environmental conditions, such as temperature and humidity with a cool dry environment being the most favourable for transmission and either warm or humid conditions being unfavourable [8]. In this study, the stability of MERS-CoV (isolate HCoV-EMC/2012) was evaluated under three different environmental conditions: high temperature and low humidity, 30°C – 30% relative humidity (RH); high temperature and high humidity, 30°C – 80% RH and low temperature and low humidity, 20°C – 40% RH, to reflect a wide range of environmental conditions including an indoor environment (20°C – 40% RH). The stability of MERS-CoV under the three tested environmental conditions was respectively compared with that of influenza A virus A/Mexico/4108/2009 (H1N1) originating from a human isolate obtained during the influenza A(H1N1) pdm09 pandemic in 2009 [9]. The stability of the two viruses in aerosols at 20°C with 40% or 70% RH was also assessed and compared.

Environmental stability

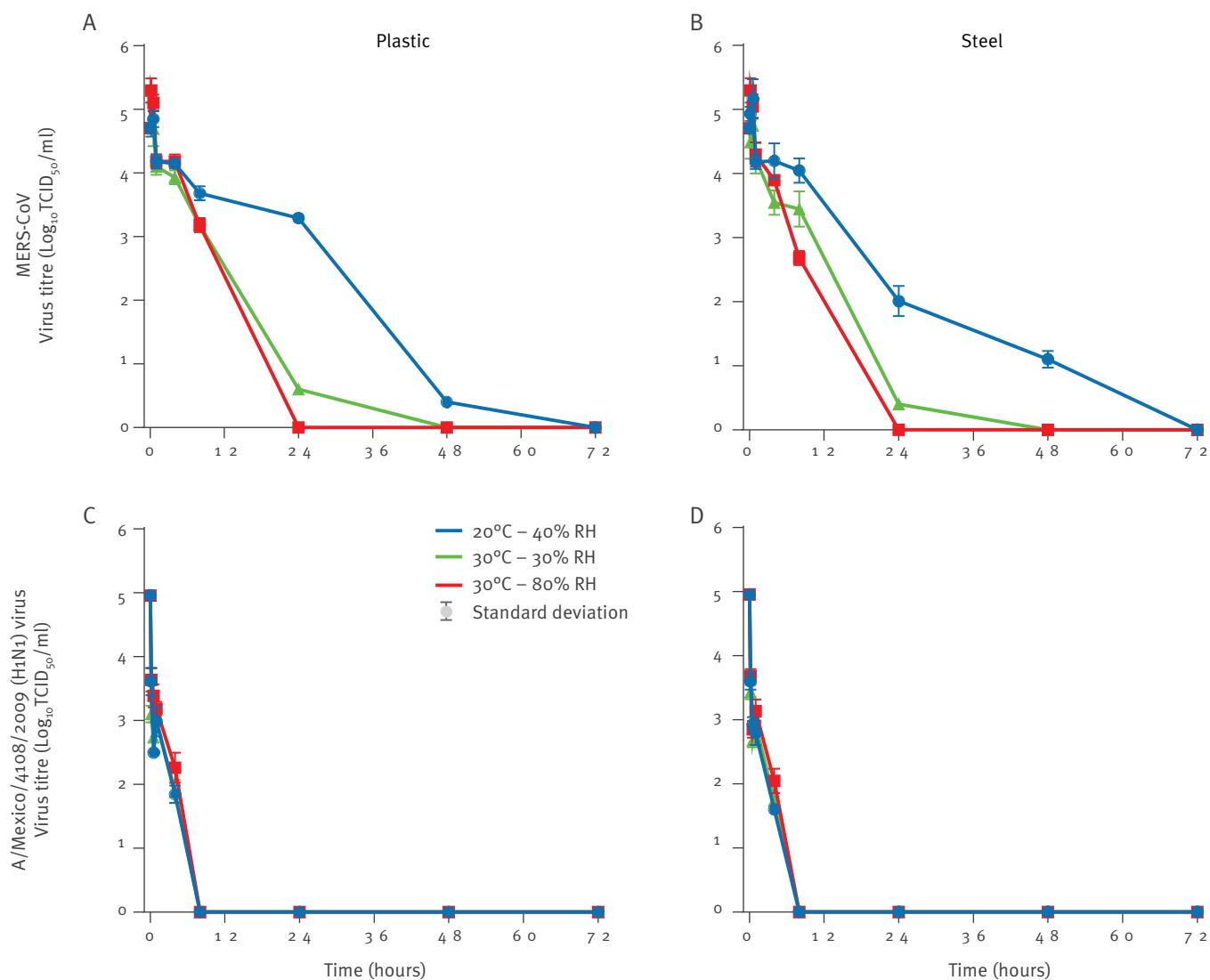
MERS-CoV (isolate HCoV-EMC/2012) and A/Mexico/4108/2009 (H1N1) virus were propagated and titrated by end-point titration on VeroE6 cells (for MERS-CoV) and Madin-Darby canine kidney (MDCK) cells (for A/Mexico/4108/2009 (H1N1) virus) as previously described [9,10]. To determine the environmental stability of the two viruses, 100 µl of 10⁶ tissue culture infective dose 50 (TCID₅₀) of MERS-CoV or A/Mexico/4108/2009 (H1N1) virus was spotted in droplets of 5 µl on the surface of steel or plastic washers (McMaster-Carr, USA) and incubated at the desired conditions in an environmental chamber (Caron, USA) for 10 and 30 minutes and 4, 8, 24, 48 and 72 hours. Experiments were conducted in triplicate. For both MERS-CoV and A/Mexico/4108/2009 (H1N1) virus, no differences in stability could be observed between the plastic and steel surface, suggesting the plastic and steel surfaces did not affect the stability differentially (Figure 1, panels A and D). MERS-CoV virus could still be recovered after 48 hours at the 20°C – 40% RH condition, whereas for the other two conditions the virus remained viable for eight (30°C – 80% RH) and 24 hours (30°C – 30% RH) respectively. For A/Mexico/4108/2009 (H1N1) virus, no virus could be recovered after four hours at each environmental condition and no difference between the environmental conditions was observed. The mean half-life values of MERS-CoV varied between 0.441822 and 0.973656 hours (five-parameter logistic model, R and R package drc [11]), but were not found to be significantly different (Table). Due to the rapid decrease in viability of A/Mexico/4108/2009 (H1N1) virus, half-life values could not be calculated.

Aerosol stability

To study their respective aerosol stability, MERS-CoV and A/Mexico/4108/2009 (H1N1) virus were aerosolised at 20°C with 40% or 70% RH (Figure 2). Aerosol experiments were performed using the AeroMP (Biaera Technologies, USA) aerosol management platform [12]. 10⁶ TCID₅₀/ml solutions of MERS-CoV and

FIGURE 1

Viability over time of Middle East respiratory syndrome coronavirus (MERS-CoV) and A/Mexico/4108/2009 (H1N1) virus under different environmental conditions



RH: relative humidity; TCID₅₀: tissue culture infective dose 50.

10⁶ TCID₅₀ of MERS-CoV (isolate HCoV-EMC/2012) (panels A and B) or A/Mexico/4108/2009 (H1N1) virus (panels C and D) was spotted on plastic (panels A and C) or steel (panels B and D) surfaces, incubated at 20°C - 40% RH (blue); 30°C - 30% RH (green) and 30°C - 80% RH (red) and titrated on VeroE6 cells (for MERS-CoV) or Madin-Darby canine kidney (MDCK) cells (for A/Mexico/4108/2009 (H1N1) virus).

A/Mexico/4108/2009 (H1N1) virus respectively were aerosolised in triplicate for 10 minutes. Aerosols were collected continuously during aerosolisation in tissue culture media (10 ml, DMEM) with an All Glass Impinger (Ace Glass Inc., USA). Collected aerosols were analysed by quantitative real-time polymerase chain reaction (qRT-PCR) and by virus titration [10,13,14]. TCID₅₀ equivalents were generated using a standard curve of 10-fold diluted MERS-CoV RNA of known concentration in the qRT-PCR. Viral genomic RNA levels in TCID₅₀ equivalents, representative of total amount of virus particles, were compared to TCID₅₀, representative of

the amount of viable virus of either MERS-CoV or A/Mexico/4108/2009 (H1N1) virus [13,14].

MERS-CoV decreased only 7% in viability at 40% RH, whereas the viability at 70% RH decreased significantly with 89% (unpaired one-tailed Student's t-test, p=0.0045). The viability of A/Mexico/4108/2009 (H1N1) virus decreased under both conditions with 95% for 40% RH and 62% for 70% RH respectively. This decrease was found to be significant at 40% RH (p=0.0095), but not at 70% RH and did not differ significantly between the two conditions.

TABLE

Decay of Middle East respiratory syndrome coronavirus (MERS-CoV) on plastic and steel surfaces at different temperatures and percent humidity

Surface type; temperature, relative humidity	Mean half-life time of MERS-CoV (hours) ^a	Standard deviation
Plastic; 20°C, 40%	0.954523	1.110443
Plastic; 30°C, 30%	0.441822	0.345291
Plastic; 30°C, 80%	0.904005	4.6838
Steel; 20°C, 40%	0.940139	1.837771
Steel; 30°C, 30%	0.973656	0.31109
Steel; 30°C, 80%	0.641163	0.825395

^a Mean half-life was determined from three independent experiments.

Discussion

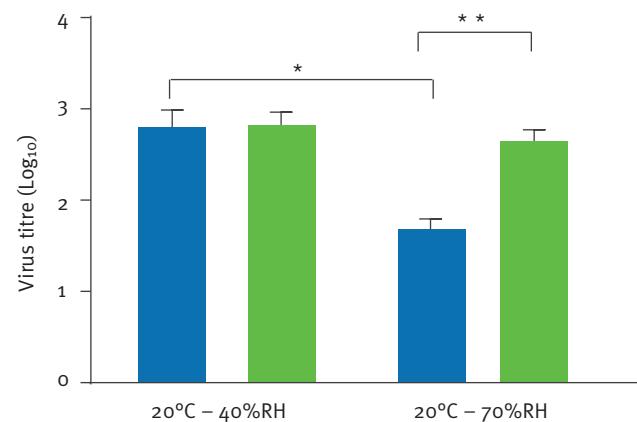
Since MERS-CoV emerged [1], an increasing number of human cases have been identified in eight different countries with a case-fatality rate of 50- to 60% [15]. Small clusters of cases with human-to-human transmission have occurred in the United Kingdom, France and Italy. In these clusters, initial cases had a recent travel history to the Middle East and subsequently infected secondary cases [2-5]. In addition, the largest cluster with suspected human-to-human transmission of MERS-CoV has been observed in Saudi Arabia and is epidemiologically linked to healthcare facilities, suggesting nosocomial transmission [16]. The recent identification of the potential circulation of MERS-CoV in dromedary camels could indicate that both zoonotic and human-to-human transmission is involved in the ongoing spread of MERS-CoV [17,18].

Here we show that compared to A/Mexico/4108/2009 (H1N1) virus, MERS-CoV remains viable for a longer duration in the environment. After four hours no viable A/Mexico/4108/2009 (H1N1) virus was detected in comparison to 8, 24 or 48 hours for MERS-CoV depending on environmental conditions (Figure 1, panels A and D). MERS-CoV was very stable in aerosol form at 20°C – 40% RH. The decrease in viability at 20°C – 70% RH (89%) was comparable to that of A/Mexico/4108/2009 (H1N1) virus. Severe acute respiratory syndrome coronavirus (SARS-CoV) has been reported to stay viable for up to five days at 22 to 25°C and 40 to 50% RH and increase in temperature and humidity resulted in a rapid loss of viability [19]. Although a comparison between different experimental studies should be approached cautiously, the relative stability of MERS-CoV at 20°C – 40% RH and the rapid decrease in virus viability at higher temperatures and higher humidity suggests that MERS-CoV and SARS-CoV share relatively similar stability characteristics. Although the route of transmission for MERS-CoV is currently unknown, the spread of MERS-CoV between people in close contact

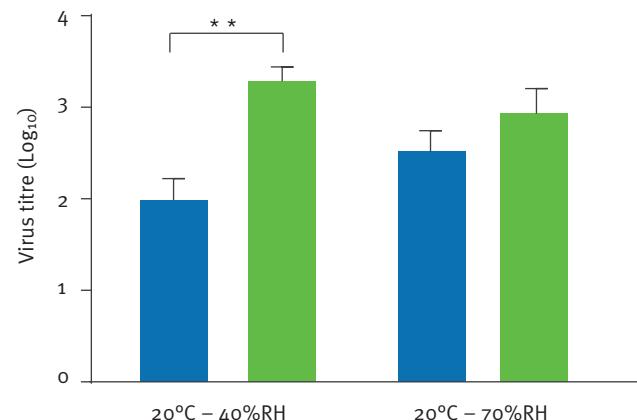
FIGURE 2

Aerosol stability of Middle East respiratory syndrome coronavirus (MERS-CoV) and A/Mexico/4108/2009 (H1N1) virus under different relative humidity conditions*

A



B



■ Virus titre in log₁₀TCID₅₀/ml
■ Virus genome copies log₁₀TCID₅₀ equivalents/ml

Error bars represent standard deviation.

* = p-value < 0.05; ** = p-value < 0.01.

TCID₅₀: tissue culture infective dose 50.

10⁶ TCID₅₀/ml of MERS-CoV (panel A) and A/Mexico/4108/2009 (H1N1) (panel B) were aerosolised and viability was determined by titration on VeroE6 cells (for MERS-CoV) or Madin-Darby canine kidney (MDCK) cells (for A/Mexico/4108/2009 (H1N1) virus), and compared to TCID₅₀ equivalents derived by quantitative real-time polymerase chain reaction (qRT-PCR).

TCID₅₀ equivalents were extrapolated from standard curves generated by adding dilutions of RNA extracted from a MERS-CoV stock with known virus titre in parallel to each run.

settings suggest contact and fomite transmission routes are most likely involved [2,3,16]. Knowledge on the environmental stability of MERS-CoV does not provide direct insights in the route of transmission; yet it does provide us with a better understanding for the potential of aerosol, contact and fomite transmission. The prolonged survival of MERS-CoV compared to A/Mexico/4108/2009 (H1N1) virus on surfaces increases the likelihood of contact and fomite transmission. However, the decrease in viability observed at high temperature suggests that direct contact transmission, and not fomite transmission, in the Arabian Peninsula would be the most likely route of zoonotic and human-to-human transmission in outdoor settings. The ability of MERS-CoV to remain viable in an airborne state suggests the potential for MERS-CoV to acquire the ability to be transmitted via aerosols. In the absence of therapeutic and prophylactic intervention strategies for MERS-CoV, a thorough understanding of the routes of transmission could be the most effective way to arrest the further spread of MERS-CoV.

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Conflict of interest

None declared.

Authors' contributions

N.v.D. and V.J.M. conceived and designed the study, N.v.D. and T.B. performed the experiments, N.v.D. and V.J.M. analysed the data, N.v.D. and V.J.M wrote the manuscript.

*Erratum

Figure 2 was corrected and replaced on 23 September 2013.

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