Preplanned Studies

Independent and Interactive Effects of Environmental Conditions on Aerosolized Surrogate SARS-CoV-2 — Beijing, China, June to September 2020

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Summary

What is already known about this topic?

Environmental factors such as temperature and humidity play important roles in the transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) via droplets/aerosols.

What is added by this report?

Higher relative humidity (61%–80%), longer spreading time (120 min), and greater dispersal distance (1 m) significantly reduced SARS-CoV-2 pseudovirus loads. There was an interaction effect between relative humidity and spreading time.

What are the implications for public health practice?

The findings contribute to our understanding of the impact of environmental factors on the transmission of SARS-CoV-2 via airborne droplets/aerosols.

Coronavirus disease 2019 (COVID-19) has led to a global pandemic and has highlighted the role of environmental factors in the transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) via droplets/aerosols. By altering the size distribution and evaporation rate of aerosols, temperature and relative humidity (RH) affect the shape and length of airborne trajectories (*1*). However, few studies have considered the interactions between multiple environmental factors and their combined impact on virus-laden droplets and aerosols.

Between June and September 2020, an orthogonal design was used to conduct suspension experiments in a 1.5 m × 1.0 m × 1.2 m laboratory exposure chamber. Independent and interactive impacts of temperature, RH, and distance on suspension time of droplets/aerosols with varying diameters and rates of size reduction of virus-laden droplets/aerosols size were explored. The numbers of droplets/aerosols with different diameters and reductions in viral load were measured in suspension and residual assays. We varied exposure chamber temperature from 16 ℃–28 ℃, RH from 30%–80%, and spreading distances of 0.5 m and 1 m to obtain data during 120 min after spreading sneeze-generated droplets/aerosols containing SARS-CoV-2 pseudovirus.

Droplets/aerosols settlement velocities increased over time under each temperature, RH, and distance range (Figure 1). With increasing time, larger aerosol particles (>1 μm) settled faster than smaller particles (<0.5 μm). After 120 min, approximately 50% of small particles (<0.5 μm) remained in suspension. Aerosol particles with diameters of >3 μm settled faster at lower RH (30%–45%), and there was a stepwise effect on aerosol particles with diameters of <0.5 μm with higher RH values (Figure 1). Aerosols remained in suspension in air currents longer than larger particles, but the numbers of suspended smaller particles decreased fastest at the highest RH range of 61%–80%.

Despite many studies on RH, few have investigated the relationship between temperature and stability of SARS-CoV-2 in aerosols. We found little difference between settling velocities of aerosols <0.5 μm in diameter under different temperature conditions compared with differences under varying RH values (Figure 1). However, particles >1 μm settled faster at higher temperatures (24 ℃–28 ℃) than at lower temperatures. Unlike variation in settling velocity from RH and temperature differences, settling velocities varied little by distances of 0.5 m and 1 m — a finding that might have been due to the relatively short (1 m) maximal dispersal distance we studied.

At the temperatures and distances studied, the lowest residual viral loads in droplets and aerosols at high RHs (61%–80%) were observed after 120 min (Table 1), suggesting that the highest RH range reduced viral loads (Figure 2A). Based on multiple

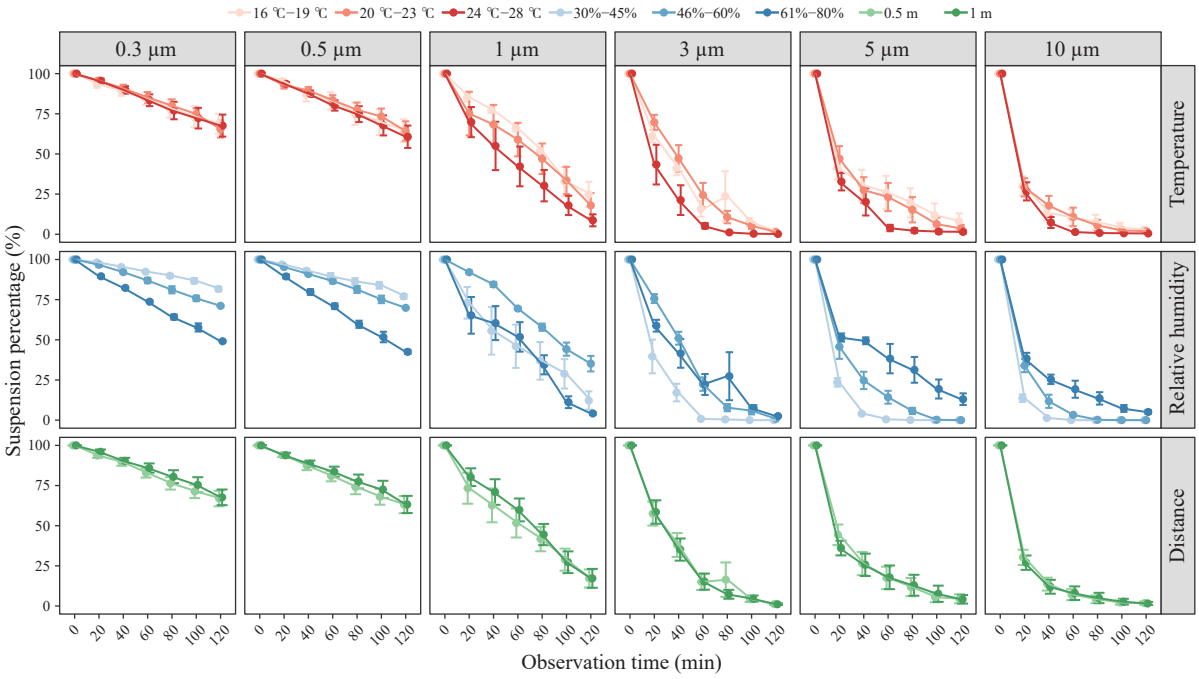

FIGURE 1. Suspension percentages of virus-laden droplet and aerosol particles with different diameters (0.3 μm, 0.5 μm, 1 μm, 3 μm, 5 μm, and 10 μm) under different conditions as a function of observation time.
Note: Environmental conditions include temperatures of 16 ℃–19 ℃, 20 ℃–23 ℃, and 24 ℃–48 ℃; relative humidity ranges of 30%–45%, 46%–60%, and 61%–80%; and spreading distances of 0.5 m and 1 m. Means and standard errors (mean±SE) are shown for three experimental replicates.

TABLE 1. Percentage of residual viral load in virus-laden droplets/aerosols under different environmental conditions at different observation time.

Experiment	T (℃)	RH (%)	Viral load (Log_{10} copies)				Percentage of residual viral load after 120 min (%)	
			0.5 m		1 m		0.5 m	1 m
			0 min	120 min	0 min	120 min	120 min *vs.* 0 min	120 min *vs.* 0 min
1	16–19	30–45	6.83	4.80	6.46	4.45	70.28	68.89
2	16–19	46–60	6.75	4.74	5.86	4.68	66.22	79.86
3	16–19	61–80	6.86	3.91	5.97	3.56	60.00	59.63
4	20–23	30–45	6.57	4.57	6.81	4.61	69.96	67.69
5	20–23	46–60	6.73	4.70	6.80	4.70	69.84	68.93
6	20–23	61–80	6.88	4.13	6.80	4.04	60.03	59.41
7	24–28	30–45	6.78	4.57	6.46	4.72	67.40	73.07
8	24–28	46–60	6.71	4.56	6.37	4.53	67.96	70.64
9	24–28	61–80	6.81	4.46	6.54	3.91	65.49	59.79

Notes: Environmental conditions include temperatures of 16 ℃–19 ℃, 20 ℃–23 ℃, and 24 ℃–48 ℃; RH ranges of 30%–45%, 46%–60%, and 61%–80%; and spreading distances of 0.5 m and 1 m.
Abbreviations: T=temperature, RH=relative humidity.

linear regression analysis, a time of 120 min and a spreading distance of 1 m significantly reduced droplet/aerosol viral loads (Figure 2A), with the most significant reduction factor being time. Mean viral loads after 120 min at distances of 0.5 m and 1 m were 66.33% and 67.81% of the mean viral loads at 0 min (Table 1).

We observed a significant interaction effect of time (120 min) and RH (61%–80%) on viral load (Figure 2C). There were no other statistically significant two-way or three-way interactions (Figure 2B, 2D, and 2E). According to modeling results, residual viral load decreased at high RH (61%–80%), while an increase in time (120 min)

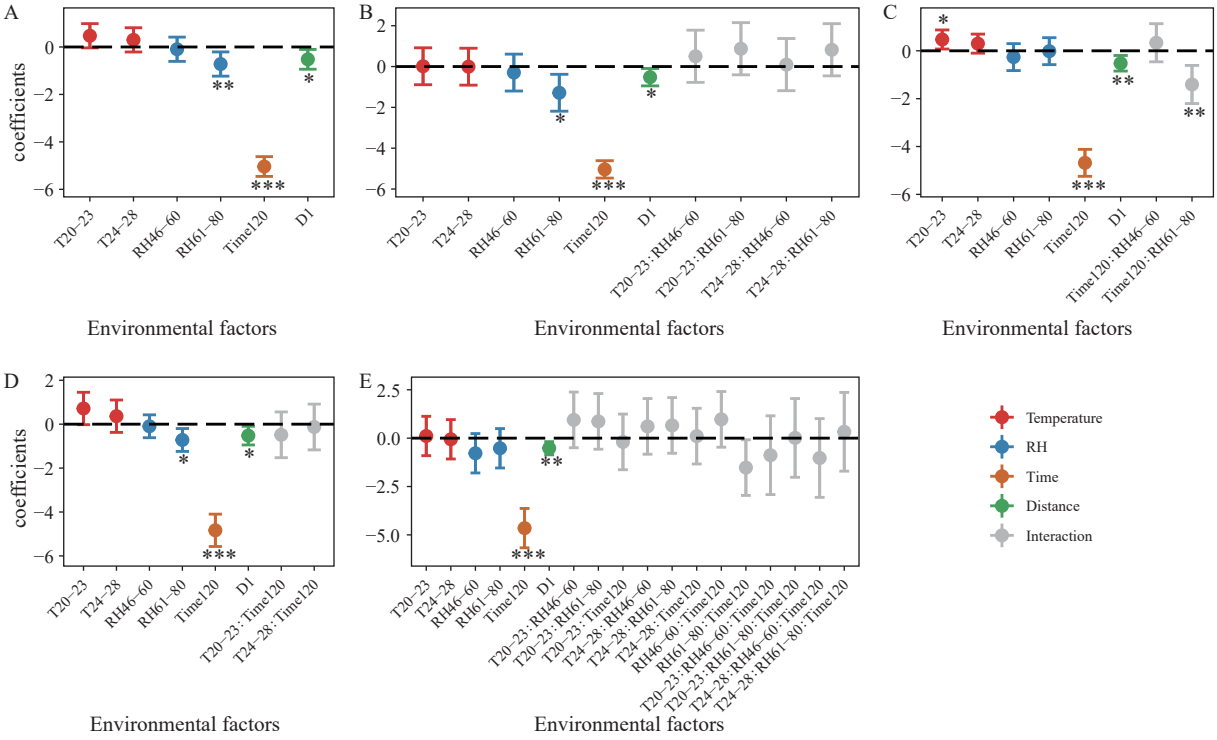

FIGURE 2. Modeled viral loads of virus-laden droplets/aerosols based on multiple interaction combinations of different environmental factors. (A) Multiple linear regression for independent factors; (B) two-way interaction between temperature and RH; (C) two-way interaction between time and RH; (D) two-way interaction between temperature and time; (E) three-way interaction among time, temperature, and RH.
Notes: Correlation refers to correlation coefficients and has no unit; T20–23 indicates the temperature was 20 ℃–23 ℃, and T24–28 indicates the temperature was 24 ℃–28 ℃; RH46–60 indicates relative humidity was 46%–60%, and RH61–80 indicates relative humidity was 61%–80%; Time120 indicates the interaction time was 120 min; and D1 indicates the spreading distance was 1 m.
Abbreviations: T=temperature, RH=relative humidity.
*: significance levels of $P<0.05$;
**: significance levels of $P<0.01$;
***: significance levels of $P<0.001$.

significantly affected the impact of RH on the viral load. Our results also showed that viral load was also significantly correlated with large particle size (≥3 μm) (Supplementary Figure S1, available in https://weekly.chinacdc.cn/), indicating that SARS-CoV-2 was mostly suspended within particles of this size class during sneezing.

DISCUSSION

The results showed that larger aerosol particles settled faster than smaller particles. The amount of small particles dicreased faster with higher relative hmidity. At high RHs, small droplets can uptake water vapor (*2*) and/or cohere to each other to form larger droplets, thus increasing their weight and size (*3*) and, therefore, increasing their settling rate. In contrast, aerosol particles with greater diameters (>3 μm) settled out faster at lower RHs (30%–45%). Higher RHs (61%–80%) significantly increased the settling velocity of aerosols with smaller diameters (<0.5 μm) and simultaneously reduced the viral load at any temperature or distance, implying that RH plays a significant role in the spread of SARS-CoV-2. The risk of transmitting SARS-CoV-2 via aerosols is higher in dry indoor environments. Therefore, this risk might be reduced by regulating the RH of indoor environments.

We also found that particles larger than 1 μm settled more rapidly at higher temperatures (24 ℃–28 ℃). High temperatures increased the evaporation of water and the conversion of respiratory droplets into aerosols. Hence, relatively high temperatures may affect large particles in a similar way that low RH values do. In addition, the mean viral loads after 120 min at different distances (0.5 m or 1 m) remained high. Time had a significant effect on viral loads, so this

finding may indicate a long suspension time and potentially long-range infection through the air (*4*). But the distances we studied (0.5 m or 1 m) had little effect on aerosol particle settlement. Thus, further studies involving larger distances are required to clarify the importance of distance on aerosol transmission.

Our findings are consistent with conclusions from other studies. Larger aerosol particles (>1 μm) settled faster, consistent with a study by Lindsley and colleagues (*5*). Approximately half of the small particles (<0.5 μm) remained suspended after 120 min. Respirable viral aerosols can linger and remain viable in air for relatively long periods (<16 h) owing to their smaller size (*6*). The number of smaller particles decreased fastest at the highest RHs. Similarly, a study of influenza virus found that exhaled respiratory droplets contributed to the propagation of influenza virus at a high RH (80%) (*7*). However, our maximum observation distance was small, and the difference in viral loads at different distances was not apparent. A previous study in hospital wards in Wuhan found that SARS-CoV-2-laden aerosols could spread over a distance of up to 4 m (*8*). A modeling simulation study reported that the maximum spreading distance of droplets could reach 6 m in an extremely cold and humid environment (*1*).

The study was subject to some limitations. First, due to bio-safety concerns, the study used a SARS-CoV-2 pseudovirus instead of SARS-CoV-2 to generate droplets and aerosols. Therefore, infectivity of the virus under different environmental conditions could not be determined. Second, the experiments were performed in a laboratory exposure chamber within a quiescent indoor environment, which was not necessarily representative of real exposure scenarios. Third, high viral loads were reported for the Delta and Omicron variants of SARS-CoV-2 (*9*), and these variants of concern (VOCs) were prone to spreading quickly in enclosed spaces (*10*). However, we did not consider the potential differences in the stabilities and transmission of these VOCs and/or variants of interest under different environmental conditions.

This study found that temperature, RH, spreading time, and dispersal distance, as well as the interaction between RH and spreading time, significantly affect the transmission of SARS-CoV-2 pseudovirus via droplets/aerosols. These findings highlighted the independent and interactive effects of environmental factors on virus-laden droplets and aerosols. By elucidating the effects of different environmental conditions on the trajectory of airborne viral transmission, adaptive public health strategies for preventing and controlling COVID-19 could incorporate seasonal weather variations and local environments. In order to reduce viral load and duration in the air, the following targeted preventive control measures might be adopted: 1) appropriately increase air humidity in residential and confined public places (e.g., using humidifiers); 2) appropriately increase ambient temperature; 3) increase the frequency of air disinfection; and 4) expand the scope of disinfection. Our study provided useful information for policymakers and guidance for the general public in the global combat against COVID-19.

Conflicts of Interest: No conflict of interest.

Acknowledgments: Special thanks to Dr. X. Shi from NIEH, China CDC for his tremendous support and guidance of this study, and Dr. S. Yang from University of South Carolina and T. Liu from South China Institute of Environmental Science for supporting on statistical analyses.

Funding: Supported by the Key Program of National Natural Science Foundation of China (No. 92043201) and the National Natural Science Foundation of China (No. 52091544), the Capital's Funds for Health Improvement and Research (No. 2021-1G-2172), the Young Scholar Scientific Research Foundation of National Institute of Environmental Health (NIEH), Chinese Center for Disease Control and Prevention (China CDC, No. 2020YSRF-03), and the COVID-19 Emergency Funding from NIEH, China CDC (No. GWTX05) and Bureau of Disease Prevention and Control, National Health Commission of China (No. WJW2102-01).

doi: 10.46234/ccdcw2022.123

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Submitted: May 06, 2022; Accepted: June 16, 2022

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SUPPLEMENTARY MATERIAL

Materials and Methods

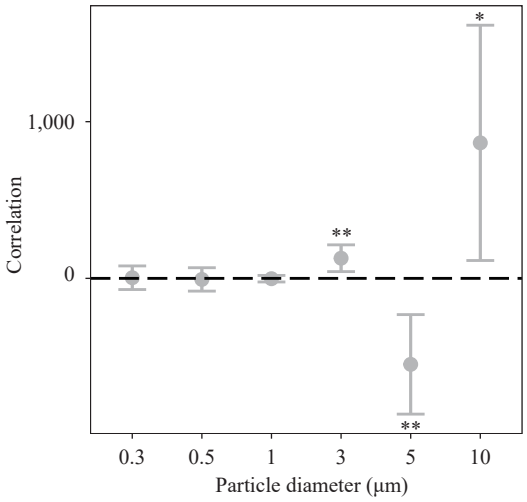
Study design: The orthogonal experiment was designed with two main aims. First, a suspension assay was used to investigate the impacts of environmental conditions on the suspension rates of pseudovirus-laden droplets and aerosols with different diameters. Second, a residual assay was used to determine the independent and interactive effects of environmental conditions on the viral loads of droplets/aerosols. The experimental conditions included temperature ranges of 16 ℃–19 ℃, 20 ℃–23 ℃, and 24 ℃–28 ℃; RH ranges of 30%–45%, 46%–60%, and 61%–80%; and spreading distances of 0.5 m and 1 m from the outlet of a sneezing simulation device. For the particle suspension assay, the observation times (i.e., durations after aerosolization) were 0 min, 20 min, 40 min, 60 min, 80 min, 100 min, and 120 min; 0 min and 120 min were selected for the residual assay. A SARS-CoV-2 spike pseudovirus (Sino Biological Inc., Beijing, China) without autonomous replication ability was used as a proxy for SARS-CoV-2 to determine the impact of each environmental factor on the viral loads of droplets/aerosols. According to a previous study (*1*), all experiments began with a similar viral concentration in artificial saliva suspensions and a similar number of sneeze-produced aerosol particles.

Experimental setup: Experiments were carried out in a laboratory exposure chamber (1.5 m × 1.0 m × 1.2 m) equipped with a high-efficiency filter to ensure the cleanliness of initial air under a quiescent environment (*2–3*). A temperature regulator (Jingchuang, RCW-360WIFI, China) and a humidity regulator (Soleusair, AHU-300N1, USA) were used to adjust the temperature and RH conditions, respectively, before each experiment according to the orthogonal experimental design. Dark conditions were maintained in the chamber throughout the experiments to avoid the potential influence of natural ultraviolet rays. We constructed a sneeze aerosol simulator comprising a compressor, an automated (on/off) electrical modulating valve, a manual electrical modulating valve, and a spray gun (*2–3*). The automated electrical modulating valve controlled the sneezing duration to 1 s. The manual electrical modulating valve adjusted the sneezing flow rate to 11±2 m/s, the total number of droplets/aerosols (diameters of 0.1 μm–100 μm) to 10^6, and the total aerosol volume to 70 μL/sneeze. To avoid cross-contamination, the chamber was ventilated with clean air [high-efficiency particulate air (HEPA)] during each test. After each test, the internal part of the chamber was wiped with 75% ethanol and then left to dry under clean air conditions.

Particle suspension assay: The poly-disperse SARS-CoV-2 pseudovirus solution was ejected 5 times (simulating 5 sneezes) from artificial saliva (10^8 copies/mL). The real-time particle number concentration (PNC) was measured with diameters ranging from 0.3 μm to >10 μm during each simulated sneeze. Particle measurement devices were set up at two different distances (0.5 m and 1 m away from the sneeze outlet). The sampling inlets of all devices were positioned along the centerline facing the sneeze outlet. At each sampling location, a Y09-301 Laser Particle Counter (AC-DC, Jiangsu Sujing Group Co., Ltd., China) was used to monitor the PNC. The data logging interval were set to 1s for all experiments. The testing times were 0 min, 20 min, 40 min, 60 min, 80 min, 100 min, and 120 min after each simulated sneeze. To determine potential variations, tests were repeated three times (n=3).

Residual assay of the viral load: Virus-laden droplets and aerosols were collected using bio-aerosol samplers (BIOSAMPLER, SKC, California, USA; sampling flow of 12.5 L/min and sampling frequency of 10 min) at 0 min and 120 min to detect the viral load. The obtained SARS-CoV-2 pseudovirus on the filter membranes was eluted with 1 mL of viral preservation medium (Dakewe Biological Engineering Co. Ltd, Shenzhen, China). Viral ribonucleic acid (RNA) was extracted using a QIAamp Viral RNA Mini Kit (QIAGEN Inc., Hilden, Germany) following the relevant protocol. The copy number of viral RNA was measured using a QX200 droplet digital polymerase chain reaction (ddPCR) system (Bio-Rad, California, USA) targeting the *WPRE* gene. Detailed information on the viral RNA extraction process, primer and probe sequences, reaction mix, droplet digital ddPCR amplification parameters, and quality assurance and quality control (QA/QC) can be found elsewhere (*2–3*).

Statistical analyses: Multiple linear regression analysis was performed using R (version 3.6.2, R core team 2021. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/) to determine the relationship between each environmental factor and the viral load. First, we examined the effects of different temperature ranges (16 ℃–19 ℃, 20 ℃–23 ℃, and 24 ℃–28 ℃), RHs (30%–45%, 46%–60%, and 61%–80%), spreading distances (0.5 m and 1 m), and observation time (0 min–



SUPPLEMENTARY FIGURE S1. Effect of the diameters of virus-laden droplets/aerosols on the viral load.
Notes: We defined a correlation greater than 0 as a positive correlation, less than 0 as a negative correlation.
*: significance levels of $P<0.05$.
**: significance levels of $P<0.01$.

120 min). A density plot was used to observe the distribution of viral load as evidence of proper transformation. The intercept of the regression model represented the base status for each environmental factor of interest: time of 0 min, distance of 0.5 m, temperature of 16 ℃–19 ℃, and RH of 30%–45%. Second, we included two-way and three-way interaction terms to account for the interactions amongst the various environmental conditions. Third, we further considered the effects of different particle diameters on the viral load. A *P*-value of 0.05 was taken as the nominal level to determine the statistical significance in all analyses.

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