INTRODUCTION

The current outbreak of the novel coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in more than 200 countries has become a serious threat to the health of people around the world. A cumulative total of over 25 million cases and 850 000 deaths have been reported so far. SARS-CoV-2 is a highly contagious virus that can transmit through respiratory droplets, aerosols, or contacts. Therefore, the stability of the virus in the environment and its activity on the surface of objects directly affect the efficiency of virus transmission. Temperature is one of the most important environmental factors affecting the stability of the virus. Studies have shown that the incidence of SARS is 18 times higher at lower air temperatures than at higher temperatures, and there is a negative correlation between the average temperature of a country and the number of cases of SARS-CoV-2 infections. A study of 24 139 positive SARS-CoV-2 cases was conducted in 26 regions in China, and the results showed that with a 1°C increase in the minimum ambient air temperature, the cumulative number of cases decreases by 0.86%. Scientific studies dealing with the viability of the virus on surfaces have confirmed that the virus can survive and maintain its infective potential for a variable period on different surfaces, and therefore this is not discussed in this study.

1 INTRODUCTION

This study was designed to investigate the sensitivity of SARS-CoV-2 to different temperatures, to provide basic data and a scientific basis for the control of COVID-19 epidemic. The virus was dispersed in 1 mL basal DMEM medium at a final concentration of 10^3.2 TCID_{50}/mL and then incubated at 4, 22, 30, 35, 37, 38, 39 and 40°C for up to 5 days. The infectivity of residual virus was titrated using the Vero E6 cell line. The results showed that the virus remained viable for 5 days at 4°C, and for 1 day only at 22 and 30°C. We found that the infectivity of the virus was completely lost after less than 12 hours at 37, 38 and 39°C, while at 40°C, the inactivation time of the virus was rapidly reduced to 6 hours. We show that SARS-CoV-2 is sensitive to heat, is more stable at lower temperatures than higher temperature, remains viable for longer at lower temperatures, and loses viability rapidly at higher temperatures.

KEYWORDS
SARS-CoV-2, sensitivity, temperature, viability
2 | METHODS

2.1 | Cells and viruses

The SARS-CoV-2 virus (SARS-CoV-2/WH-09/human/2020/CHN; GenBank: MT093631.2) was isolated by ILAS, PUMC. Vero E6 cells were used for the reproduction of SARS-CoV-2 stocks. Vero E6 cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum, 100 IU/mL penicillin, and 100 μg/mL streptomycin, and incubated at 37°C, in 5% CO₂. Titers for SARS-CoV-2 were resolved by a 50% tissue-culture infectious doses (TCID₅₀) assay.

2.2 | Viral titer

The 10-fold diluted virus droplets were inoculated into simple Vero E6 cells and incubated at 37°C for 1 hour. The cells were then added to 200 μL DMEM medium with 2% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin, and incubated at 37°C, in 5% CO₂ for 3 days. The observed cytopathic effect and tissue culture infective dose (TCID₅₀) were calculated by the Reed–Muench method.⁸

2.3 | Comparison of stability of SARS-CoV-2 at different temperatures

Eight temperature groups (4, 22, 30, 35, 37, 38, 39 and 40°C) were set for the experiment, with 5 culture dishes in each group. The virus was dispersed in medium at a final concentration of 10².2 TCID₅₀/mL and then 2 mL virus droplets were added to each dish. In the groups at 4, 22, 30 and 35°C, virus droplets from each dish were gathered at 0, 1, 5 and 7 days for viral titer. In the groups at 37, 38, 39 and 40°C, virus droplets were gathered at 0, 6, 12, 20 and 24 hours for viral titer. Temperature was the only variable in the experiment; ambient humidity and wind speed remained basically the same. The experiment was repeated 3 times.

2.4 | Statistical analysis

One-way analysis of variance (ANOVA) and Bonferroni correction were used to evaluate the divergence of virus titers among different groups. The variance between the two groups was analyzed by Student’s t test using SPSS 11.5. Results were considered significant for P < .05.

3 | RESULTS

3.1 | Comparison of the stability of SARS-CoV-2 at different temperatures

SARS-CoV-2 at a concentration of 10².2 TCID₅₀/mL was incubated at 4°C, and virus titers were measured on different days. The virus titers detected on days 1, 3, and 5, were 10¹.64 TCID₅₀/mL, 10¹.40 TCID₅₀/mL, and 10¹.57 TCID₅₀/mL, respectively. When the incubation temperature was changed to 22°C or 30°C, the virus titers were 10² TCID₅₀/mL and could only be measured on day 1; on day 3 they were below the assay detection limit. At 35°C, the virus titers were below the detection limit on day 1 (Figure 1A).

When the virus was incubated at 37, 38 or 39°C, the virus titers could be detected at 6 hours and 12 hours but not at 20 hours. At 6 hours, the virus titers were, respectively, 10¹.87 TCID₅₀/mL, 10¹.78 TCID₅₀/mL and 10¹.67 TCID₅₀/mL. At 12 hours, the titers were, respectively, 10¹.36 TCID₅₀/mL, 10¹.29 TCID₅₀/mL and 10¹.20 TCID₅₀/mL. At 20 hours, the virus titers were too low to detect. For the virus incubated at 40°C, the virus titers were 10¹.57 TCID₅₀/mL at 6 hours and below the detection limit at 12 hours (Figure 1B).

4 | DISCUSSION

It is well known that viruses are capable of long-term survival at below 0°C, but the duration of their activity remains unknown at 4°C or at different room temperatures. Therefore the sensitivity of the virus to different temperatures (4-40°C) was tested in this study.
In present study, the virus was dispersed in 2 mL basal DMEM medium at a final concentration of $10^{3.2}$ TCID$_{50}$/mL and then incubated at 4-40°C for up to 7 days. The infectivity of residual virus was titrated using the Vero E6 cell line, and the results showed that the virus was stable at 4°C and infectivity was lost completely after 1 day at 35°C. Subsequently, the stability of the virus at higher temperatures was analyzed in detail. We found that the infectivity of the virus was completely lost after less than 12 hours at 37°C, and when the temperature was increased to 40°C, the inactivation time of virus was rapidly reduced to 6 hours. A previous study indicated that the stability of SARS-CoV-2 correlated with temperature between 4 and 70°C, as significant differences in infectivity of the virus were observed with incubation at 4, 22, 37, 56 and 70°C, with the activity of the virus being lost at 70°C after no more than 5 minutes. The present study focused on temperatures from 25 to 40°C, and we found that the virus did not survive long at temperatures of 30-40°C. Infectivity was lost within 1 day and the virus was inactivated after no more than 12 hours when the temperature was higher than 40°C. It should be noted that the viral titer we used was $10^{3.2}$ TCID$_{50}$/mL, and a different initial viral titer might yield different results for the durations of viral stability. In real-life conditions the virus titer released into the environment is random, so viral inactivation times will different from the results we obtained.

This study examines virus activity at different temperatures, providing a preliminary demonstration of the changes in viral activity with temperature and the biological characteristics of COVID-19. Our results generate further scientific data to support a greater understanding of the virus and formulation of effective prevention and control strategies.

**ACKNOWLEDGMENTS**

This work was supported by CAMS Innovation Fund for Medical Sciences (CIFMS) (No. 2016-I2M-1-014 and 2020-I2M-CoV19-009)

**CONFLICT OF INTEREST**

None.

**ORCID**

Qi Lv https://orcid.org/0000-0003-2102-2877

**REFERENCES**

1. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395(10223):497-506.
2. Wu Y, Jing W, Liu J, et al. Effects of temperature and humidity on the daily new cases and new deaths of COVID-19 in 166 countries. *Sci Total Environ*. 2020;10(729):139051.
3. Lin K, Fong DYT, Zhu B, Karlberg J. Environmental factors on the SARS epidemic: air temperature, passage of time and multiplicative effect of hospital infection. *Epidemiol Infect*. 2006;134(2):223-230.
4. Sobral MFF, Duarte GB, da Penha Sobral AIG, Marinho MLM. de Souza Association between climate variables and global transmission of SARS-CoV-2. *Sci Total Environ*. 2020;10(729):138997.
5. Eslami H, Jalili M. The role of environmental factors to transmission of SARS-CoV-2 (COVID-19). *AMB Express*. 2020;10(1):92.
6. FernándezRaga M, Díaz-Marugán L, García Escolano M, Bort C, Fanjul V. SARS-CoV-2 viability under different meteorological conditions, surfaces, fluids and transmission between animals. *Environ Res*. 2020;192:110293.
7. van Doremalen N, Bushmaker T, Morris DH, et al. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. *N Engl J Med*. 2020;382(16):1564-1567.
8. Bao L, Deng W, Huang B, et al. The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice. *Nature*. 2020;583(7818):830-833.
9. Chin AWH, Chu JTS, Perera MRA, et al. Stability of SARS-CoV-2 in different environmental conditions. *medRxiv*. 2020;3(15):20036673.

**How to cite this article:** Lv Q, Liu M, Qi F, et al. Sensitivity of SARS-CoV-2 to different temperatures. *Anim Models Exp Med*. 2020;3:316–318. https://doi.org/10.1002/ame2.12141