Prolonged Infectivity of SARS-CoV-2 in Fomites

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DOI: https://doi.org/10.3201/eid2609.201788

We spotted severe acute respiratory syndrome coronavirus 2 on polystyrene plastic, aluminum, and glass for 96 hours with and without bovine serum albumin (3 g/L). We observed a steady infectivity (<1 log_{10} drop) on plastic, a 3.5 log_{10} decrease on glass, and a 6 log_{10} drop on aluminum. The presence of proteins noticeably prolonged infectivity.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread worldwide, demonstrating a great potential for direct and indirect transmission between humans. Coronaviruses can keep their infectivity in fomites and thus can remain infectious on dry surfaces for hours (*1*,*2*). However, limited data are available for SARS-CoV-2 (*1*). Specifically, there are no data about the role of interfering substances such as proteins on SARS-CoV-2 infectivity in the environment. We evaluated the stability and infectivity of SARS-CoV-2 deposited on polystyrene plastic, aluminum, and glass for 96 hours at 45%–55% relative humidity (recommended for indoor living spaces by the American Society of Heating, Refrigeration and Air Conditioning Engineers) and 19°C –21°C temperature range using a 10^6 50% tissue culture infectivity dose ($TCID_{50}$)/mL inoculum.

We inoculated SARS-CoV-2 at a multiplicity of infection of 0.001 onto Vero E6 cells incubated at 37°C in 5% CO_2 for 72 h (Appendix, https://wwwnc.cdc.gov/EID/article/26/10/20-1788-App1.pdf). We collected the supernatant and clarified it by spinning at 1500 × *g* for 10 min. We prepared aliquots and stored them at -80°C before titration. We measured virus infectivity using $TCID_{50}$. We diluted the inoculum in cell culture medium containing 5% fetal bovine serum (FBS; final protein concentration 1.8 g/L) to 10^6 $TCID_{50}$/mL. For experiments with a higher protein concentration, we used a concentrated bovine serum albumin (BSA) solution (40 g/L) to result in a final protein concentration of 11.4 g/L. We measured virus infectivity sequentially on polypropylene plastic, aluminum, and glass slides. We deposited a 50-μL drop in triplicate on the various surfaces (≈1 cm^2 per piece) and recovered them sequentially to quantify viable infectious virions by endpoint titration on Vero E6 cells. The limit of detection for the assays was $10^{0.5}$ $TCID_{50}$/mL.

We conducted our experiments with and without BSA to mimic the protein content within body fluids of the respiratory system such as cough droplets, sputum, and airway mucosal secretions (*3*). Final protein concentration was 1.8 g/L without BSA conditions and 11.4 g/L with BSA conditions. We observed 3 different profiles, depending on surface type: a 3.5 log_{10} decrease over 44 h on glass (Figure 1, panel A), a steady infectivity with a <1 log_{10} drop over 92 h on polystyrene plastic (Figure 1, panel B), and a sharp 6 log_{10} drop in <4 h on aluminum (Figure 1 panel C). The probable adsorption of viral particles onto a plastic polystyrene surface was associated with prolonged infectivity, whereas a high drop on aluminum was observed as in previously published data on SARS-CoV, adenovirus, or poliovirus (*4*,*5*). Our results have also shown higher stability for SARS-CoV-2 on polystyrene plastic, with or without BSA, in comparison with a recent study (*1*); this variation could be explained by a different type of plastic used in the 2 studies. Regardless of the type of surface, virus infectivity decreased ≈1 log_{10} within 2 h (Table). To study SARS-CoV-2 stability in solution, we titrated cell culture supernatants containing 10^6 $TCID_{50}$/mL every 24 h for 96 h. We found that SARS-CoV-2 was very stable, showing an overall decreased infectivity <1.4 log_{10} reduction, results similar to those described for SARS-CoV (Appendix Figure) (*4*).

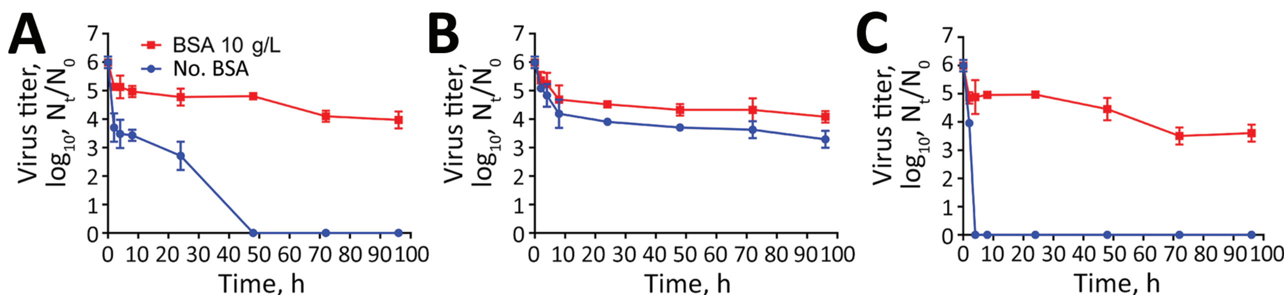

Figure. Viability of severe acute respiratory syndrome coronavirus 2 on various surfaces and in suspension. Viruses were applied to glass (A), polystyrene plastic (B), and aluminum (C) at 45%–55% relative humidity at 19°C–21°C for 96 h. The titer of viable virus is expressed as $TCID_{50}$/mL of collection medium. All samples were quantified by endpoint titration on Vero E6 cells with a limit of detection of ≈$10^{0.5}$ $TCID_{50}$/mL. $TCID_{50}$, 50% tissue culture infectivity dose.

Table. SARS-CoV-2 titer values for different materials*

	Material						
	Glass		Aluminum		Plastic		SARS-CoV-2 in suspension
Time, h	No BSA	BSA 10 g/L	No BSA	BSA 10 g/L	No BSA	BSA 10 g/L	
0				6 ± 0.2			
2	3.7 ± 0.5	5.1 ± 0.1	4 ± 0.1	4.8 ± 0.2	5.1 ± 0.1	5.4 ± 0.3	
4	3.5 ± 0.5	5.1 ± 0.4	ND	4.8 ± 0.5	4.8 ± 0.4	5.2 ± 0.4	
8	3.4 ± 0.2	4.9 ± 0.2	ND	4.9 ± 0.1	4.2 ± 0.5	4.6 ± 0.5	
24	2.7 ± 0.5	4.7 ± 0.3	ND	4.9 ± 0.1	3.8 ± 0.1	4.5 ± 0.1	5.99
48	ND	4.8 ± 0.1	ND	4.4 ± 0.4	3.7 ± 0.1	4.3 ± 0.2	4.99
72	ND	4.1 ± 0.2	ND	3.4 ± 0.3	3.6 ± 0.3	4.3 ± 0.4	3.99
96	ND	3.9 ± 0.3	ND	3.6 ± 0.3	3.3 ± 0.3	4.1 ± 0.2	3.99
Half-life	17	>96	2.5	>96	>96	>96	>96

*Values are mean value of 3 replicates ± SD. BSA, bovine serum albumin; ND, not detectable; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Our data showed that SARS-CoV-2 infectivity was remarkably preserved in the presence of proteins, regardless of the type of surface. A final concentration of 11.4 g/L of proteins, as used in our study, closely mimics that of respiratory fluids, which possess protein concentrations of a similar order of magnitude. However, the respiratory body fluids are complex media including not only proteins, but also enzymes and mucins (present in mucus) that may have a negative effect on virus infectivity. Regarding viral load measurement, the reason for avoiding the use of molecular techniques such as reverse transcription PCR is that despite that they allow quantification of RNA copies and determination of RNA decay, they cannot measure residual infectivity on various surfaces.

The protective effect of proteins had already been described for pandemic SARS-CoV or suggested for influenza A(H1N1) virus, but with less notable effects (*4*,*6*). As illustrated in other virus models (*7*), interfering substances such as proteins influenced the resistance of SARS-CoV-2 to drying and thus its persistence in the environment.

In conclusion, we showed that a moderate protein concentration in droplets markedly increased the infectivity of SARS-CoV-2, suggesting that a protein-rich medium like airway secretions could protect the virus when it is expelled and may enhance its persistence and transmission by contaminated fomites. Accordingly, it is plausible that fomites infected with SARS-CoV-2 play a key role in the indirect transmission of coronavirus disease (COVID-19). This finding supports surface cleaning as a necessary action that should be enforced and repeated becuase it may play a key role in halting SARS-CoV-2 transmission and mitigating the COVID-19 pandemic.

This study was funded in part by the European Virus Archive Global (EVA-GLOBAL) project H2020-INFRAIA-2019 program, project no. 871029, Preparedness and Response in an Emergency Contact to Pathogens of Medical and Veterinary Importance (PREPMedVet), Agence Nationale de la Recherche Franco-German Call on Civil Security/Global Security 2019 Edition, and the Inserm through the Reacting (REsearch and ACTion Targeting Emerging Infectious Diseases) initiative.

About the Author

Dr. Pastorino is research engineer with a PhD in virology. His primary research interests are biosafety aspects of Biosafety Level 3 viruses in the context of preparedness and response against epidemics.

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Article DOI: https://doi.org/10.3201/eid2609.201788

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Appendix

Methods

Cell Line

Vero E6 cells (ATCC#CRL-1586) were grown at 37°C in 5% CO_2 with 1% penicillin/streptomycin (PS; 5000 U/mL and 5000 μg/mL; Life Technologies, https://www.thermofisher.com/us/en/home/brands/life-technologies.html) and supplemented with 1% nonessential amino acids (Life Technologies) in minimal essential medium (MEM; Life Technologies) with 5% FBS.

Viruses

The human 2019 SARS-CoV-2 strain (Ref-SKU: 026V-03883) was isolated at Charite University (Berlin, Germany) and obtained from the European Virus Archive catalog (EVA-GLOBAL H2020 project) (https://www.european-virus-archive.com). Experiments were performed in Biosafety Level 3 (BSL3) facilities.

SARS-CoV-2 Titration

SARS-CoV-2 was first propagated and titrated on Vero E6 cells. Virus stock was diluted to infect Vero E6 cells at a multiplicity of infection (MOI) of 0.001, then cells were incubated at 37°C for 24–48 h, after which medium was changed and incubation was continued for 24 h; then supernatant was collected, clarified by spinning at 1500 × *g* for 10 min, supplemented with 25 mM HEPES (Sigma, https://www.sigmaaldrich.com), and aliquoted. Aliquots were stored at −80°C before titration. Virus infectivity was measured using 50% tissue culture infectivity dose ($TCID_{50}$); briefly, when cells were at 90% confluence, 6 replicates were infected with 150 μL of tenfold serial dilutions of the virus sample and incubated for 4 days at 37°C under 5% CO_2. Cytopathic effect was read using an inverted microscope, and infectivity was expressed as $TCID_{50}$/ml based on the Karber formula (*1*).

Virus Stability on Surfaces

Surface stability was evaluted on plastic (polypropylene; Corning Inc., https://www.corning.com), aluminium (Thermo Scientific, https://www.thermofisher.com), and glass (Thermo Scientific). All experiments were conducted in a BSL-3 lab under biosafety cabinet at 45–55% relative humidity and 19–21°C. An inoculum of 10^6 $TCID_{50}$/mL was resuspended in cell culture medium (complete MEM plus 5% FBS) with or without BSA 10 g/L and placed on test surfaces. In short, 50 µL of virus was deposited on the surface (≈1 cm^2 per piece) and recovered at predefined timepoints by adding 150 µL of complete medium. Three replicate experiments were performed for each surface and viable virus was immediately quantified by endpoint titration on Vero E6 cells, as described previously (*2*). The limit of detection for the assays was about $10^{0.5}$ $TCID_{50}$/mL.

Results

In suspension, SARS-CoV-2 was stable for the entire duration of the experiment (96 h) (virus titer decrease <1.4 log_{10}) as already described for SARS coronavirus (Appendix Figure) (*3*).

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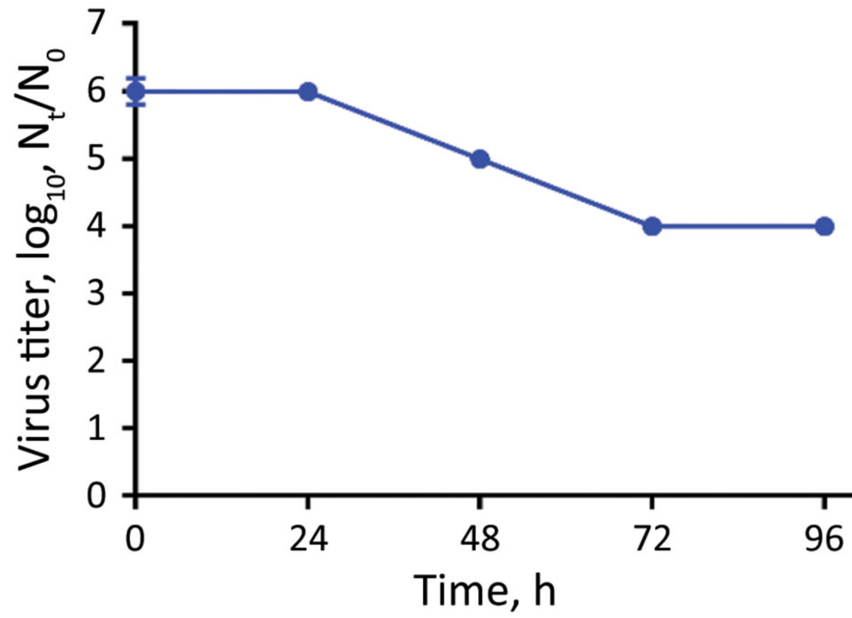

Appendix Figure. In vitro stability of SARS-CoV-2. Infected cell supernatant (complete MEM medium, 5% FCS) was incubated at room temperature in suspension and titrated every 24 hours. Values are means for 2 independent experiments.