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BRIEF REPORT

Stability and Inactivation of Monkeypox Virus on Inanimate Surfaces

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The spread of nonzoonotic monkeypox virus (MPXV) infections necessitates the reevaluation of hygiene measures. To date, only limited data are available on MPXV surface stability. Here, we evaluate the stability of infectious MPXV on stainless steel stored at different temperatures, while using different interfering substances to mimic environmental contamination. MPXV persistence increased with decreasing temperature. Additionally, we were able to show that MPXV could efficiently be inactivated by alcohol- and aldehyde-based surface disinfectants. These findings underline the stability of MPXV on inanimate surfaces and support the recommendations to use alcohol-based disinfectants as prevention measures or in outbreak situations.

Keywords. stability; MPXV; disinfection; inactivation; inanimate surface.

Human monkeypox virus (MPXV) outbreaks spread internationally in 2022, causing concern worldwide [1, 2]. The main transmission route for MPXV involves close and prolonged skin-to-skin contact, especially direct contact with infected body fluids or infectious lesions (rash, blisters, pustules, wounds, scabs) [3]. Although documented occurrences are rare, transmission can also occur by direct contact with objects or materials (fomites) contaminated with MPXV [4, 5]. During the current outbreak, widespread surface contamination has been described in different scenarios, including households and in hospital rooms of people with symptomatic MPXV infections [6–8] and MPXV has been detected on household surfaces at least 15 days after contamination of the surfaces [9]. Hence, evaluating the survival and inactivation kinetics of MPXV on surfaces is important to define precise measures for minimizing viral transmission during the ongoing MPXV outbreak. However, so far only limited data are available on surface stability of MPXV. Moreover, in order to adequately assess the risk of transmission for a given pathogen and to determine appropriate hygiene measures, detailed information about its susceptibility toward disinfectants is required. Given the long-lasting surface stability of other viruses of the *Orthopoxvirus* genus [10, 11], it is particularly important to confirm which disinfectants and biocidal agents are able to inactivate MPXV. To date, only limited data are available on inactivation efficacies of different disinfectants [12].

Here, we evaluated the stability of infectious MPXV on stainless steel discs stored at different temperatures in combination with different interfering substances to mimic different environmental conditions and further evaluated the virucidal activity of different commercially available surface disinfectants.

METHODS

Cell Culture and MPXV Production

Vero76 cells were cultured in Dulbecco modified Eagle medium supplemented with 10% (vol/vol) fetal calf serum (FCS), 1% (vol/vol) nonessential amino acids, 100 IU/mL penicillin, 100 µg/mL streptomycin, and 2 mmol/L L-glutamine. Test suspensions with MPXV (MPXV-DUS_001, classified as MPXV subgroup II) were prepared as discribed recently [12].

MPXV Stability Testing

Stainless steel discs (2-cm-diameter discs, article number 4174–3000, GK Formblech GmbH, Berlin, Germany) were decontaminated in 70% (vol/vol) ethanol for 15 minutes and contaminated with 50 µL virus solution containing 9 parts MPXV and 1 part interfering substance (bovine serum albumin [BSA] or sheep blood). Immediately after, one-third of contaminated steel discs were either placed at 4 °C, 22 °C, or 37 °C. Virus was recovered at different time points (15, 30, 45, 60, 120, and 240 minutes, every 24 hours between 1 and 10 days, and every 2–3 days after 10 days of incubation) postcontamination by transferring 3 steel discs of each condition into a 25 mL container harboring 2 mL of cell culture medium (without FCS) and subsequent vortexing. An end-point dilution assay was performed on Vero76 cells to determine the remaining infectivity.

Surface Disinfectants

Five different commercially available surface disinfectants (products I–V) were tested regarding their potential to

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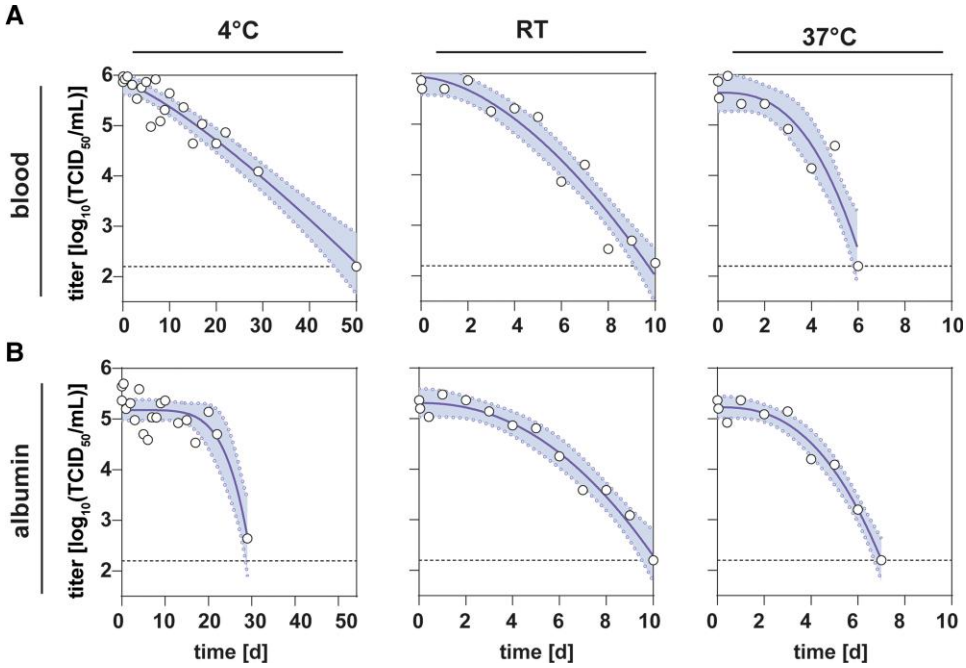

Figure 1. Stability of monkeypox virus (MPXV) on steel discs at 4 °C, room temperature (RT), or 37 °C. Recovered MPXV, mixed with sheep blood (*A*) or bovine serum albumin (*B*) as an interfering substance, displayed as 50% tissue culture infectious dose ($TCID_{50}$)/mL (y-axes) over time (continuous x-axes). Dots represent technical replicates, purple lines and shaded areas display the course of the Weibull distribution–fitted data and 95% confidence interval, and dashed lines represent the limit of detection.

inactivate MPXV. Depending on their active compounds (Supplementary Table 1), the disinfectants can be subdivided into (1) alcohol-based products: product I (Bacillol AF, 450 mg/g 1-propanol, 250 mg/g 2-propanol, 47 mg/g ethanol) and product II (Antifect N liquid, 250 mg/g ethanol, 350 mg/g 2-propanol); (2) aldehyde-based products: product III (Kohrsolin FF, 50 mg/g glutaraldehyde, 30 mg/g benzylalkyldimethylammonium chloride, 30 mg/g didecyldimethylammonium chloride) and product IV (Incidin Rapid, 98 mg/g glutaraldehyde, 50 mg/g alkyldimethylbenzylammonium chloride, 50 mg/g didecyldimethylammonium chloride); and (3) hydrogen peroxide–based products: product V (Incidin OxyFoam, 15 mg/g hydrogen peroxide).

MPXV Inactivation by Surface Disinfectants

Stainless steel discs were decontaminated as mentioned above. Subsequently, the stainless steel discs were contaminated with 50 µL virus solution containing 9 parts MPXV and 1 part interfering substance (BSA, final concentration 0.3 g/L, clean condition). After visible drying of the inoculum, 100 µL of surface disinfectant was applied onto the carrier and incubated at indicated concentrations for indicated time periods at room temperature (Supplementary Table 1). Hereafter, the specimens were transferred into a 25-mL container harboring 2 mL cell culture medium (without FCS) and vortexed for 60 seconds to resuspend the virus. An end-point dilution assay was performed on Vero76 cells to determine the remaining infectious viral titers. After 7 days, cytopathic effects were evaluated microscopically and used to calculate the 50% tissue culture infectious dose ($TCID_{50}$)/mL.

RESULTS

MPXV Stability on Stainless Steel Discs at Different Temperatures

Here, we evaluated the surface stability of MPXV. Stainless steel discs were inoculated with virus solution containing either BSA or sheep blood as interfering substance and the residual infectivity was monitored over time at 4 °C, 22 °C, and 37 °C. A fitted Weibull distribution model was employed to determine initial decay rates and model time to lower limit of quantification. The initial virus concentration of 2.72×10^5 $TCID_{50}$/mL remained constant within the first 24 hours independent of the temperature or interfering substance (Figure 1*A* and 1*B*). After 5 days, initial viral titers declined upon incubation at 22 °C and 37 °C for either sheep blood and BSA. Upon incubation at 37 °C, no infectious virus could be recovered after day 6 (sheep blood) and day 7 (BSA), matching decay times ($t_{1/2}$) of 4.82 days and 5.36 days, respectively. Likewise, upon incubation at 22 °C, no infectious virus could be recovered after day 10 (BSA) and day 11 (sheep blood), matching $t_{1/2}$ of 7.38 days and 6.47 days, respectively. In contrast, upon incubation at 4 °C, infectious virus could be recovered up to 30 days. Virus solutions containing BSA started to strongly decline after day 20 ($t_{1/2} = 26.37$ days), while viral titers of the solution

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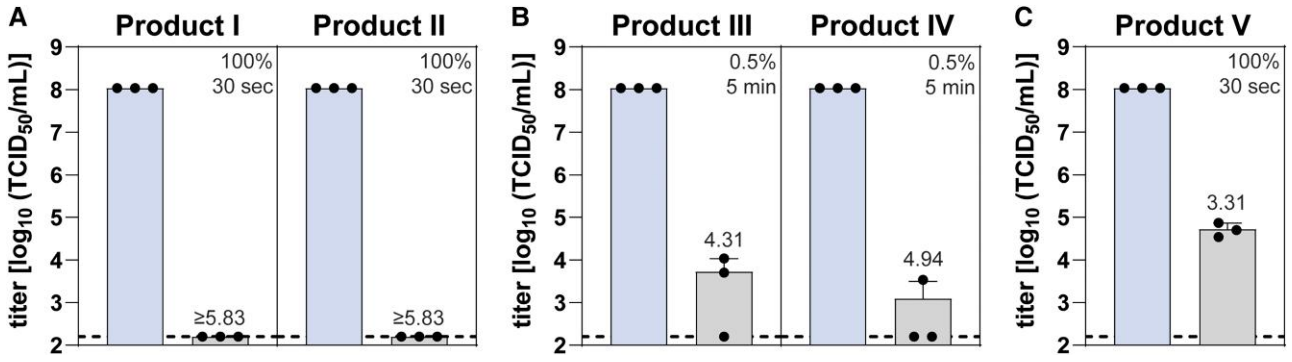

Figure 2. Susceptibility of monkeypox virus (MPXV) to surface disinfectants. Effect of products against MPXV: 2 alcohol-based disinfectants, product I and product II (*A*); 2 aldehyde-based disinfectants, product III and product IV (*B*); and 1 hydrogen peroxide–based disinfectant, product V (*C*). Products were tested at concentrations according to the manufacturers' instructions. Cell culture medium was used as control. Bars represent infectious titer, and dotted lines represent the lower limit of quantification (158 $TCID_{50}$ [50% tissue culture infectious dose]/mL) (n = 3, mean ± standard deviation). Reduction factors are displayed vertically for the product-treated conditions.

containing sheep blood declined earlier, but more evenly over the observed time frame ($t_{1/2}$ = 29.04 days).

Overall, the surface stability of MPXV was highly temperature dependent, while comparable inactivation kinetics between virus solution containing either BSA or sheep blood as interfering substances were observed.

MPXV Inactivation by Commercially Available Surface Disinfectants

Given the high surface stability of MPXV, we next evaluated 5 different commercially available surface disinfectants (Supplementary Table 1) according to the manufacturers' recommendations. Ready-to-use alcohol-based disinfectants (products I and II) efficiently inactivated MPXV with a reduction factor (RF; log_{10} $TCID_{50}$/mL) ≥5.83 (Figure 2*A*). Although infectious viral titers were not reduced to the lower limit of detection, 2 aldehyde-based disinfectants (product III and IV) still efficiently lowered MPXV infectivity, corresponding to an RF ≥4.31 (product III) and RF ≥4.31 (product IV) (Figure 2*B*). In contrast, MPXV was only moderately inactivated upon application of a hydrogen peroxide–based disinfectant (product V) and only displayed an RF ≥3.31 (Figure 2*C*). Despite its high surface stability, MPXV could be efficiently inactivated by alcohol- and aldehyde-based products (products I–IV), whereas the hydrogen peroxide-based product (product V) did not efficiently inactivate the virus under the tested conditions.

DISCUSSION

The risk of viral transmission from a surface depends on the contact frequency, time, body parts, and how readily the virus is released from such surfaces. Poxvirus virions are known for their long-term environmental persistence [10, 11]. In agreement with this, we observed that MPXV virons could be recovered from stainless steel discs for several days, including up to 30 days when stored at 4 °C. The surface stability of MPXV on stainless steel was highly temperature-dependent, while more or less comparable inactivation kinetics between virus solution containing either BSA or sheep blood as interfering substances were observed at room temperature and 37 °C. In line with our observations of MPXV survival for 10–11 days at 22 °C, infectious MPXV was recently reported to persist in a household environment for >15 days at ambient temperatures [9]. Poxvirus virions shed during infection within lesion are known to be more resistant to desiccation due to tight binding to fibrin matrices of the scab/crust material. This might contribute to maintain viral infectivity over time in real-life scenarios [3, 8]. Hence, the high surface stability of MPXV necessitates appropriate hygiene measures to lower the risk of transmission.

We therefore tested 5 different commercially available surface disinfectants according to the manufacturers' recommendation for their ability to inactivate MPXV on stainless steel. In agreement with recent findings from quantitative suspension tests [12], MPXV deposited on stainless steel surfaces could efficiently be inactivated using alcohol-based disinfectants. Likewise, aldehyde-based disinfectants efficiently inactivated MPXV, whereas the tested hydrogen peroxide–based product did not efficiently inactivate the virus under the tested conditions missing a 4 log_{10} reduction. Improved hygiene measures for patients with an ongoing MPXV infection will allow to minimize its spread, especially in cases of outbreaks. It should be considered that in our study only stainless steel carriers were used to test persistence and stability against surface disinfectants, and future studies should also examine MPXV stability on other materials. Moreover, additional environmental factors such as viral load, humidity, and solar radiation were not considered in our controlled laboratory setting and might further influence MPXV surface stability and thus influence surface transmission. Nonetheless, our findings support the current guidelines of the World Health Organization and national

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health authorities and underscore the need for and timely application of alcohol-based disinfectants as an effective measure for minimizing viral transmission and maximizing viral inactivation during the ongoing MPXV outbreak.

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. The 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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