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Efficacy of Laundry Practices in Eliminating Mpox Virus From Fabrics

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Background. The declaration of mpox as a public health emergency of international concern highlights the need for interventions to interrupt virus transmission, including transmission via fabrics. Current World Health Organization (WHO) guidance on clothes washing is based on a general consensus of virus inactivation; however, there is uncertainty about the efficacy of laundry detergents and disinfectants or the reduction of risk achieved by washing clothes for mpox virus (MPXV) specifically.

Methods. This study investigates the efficacy of manual washing for inactivating MPXV from clothes. Using a simulated washing method, we evaluated the efficacy of commonly used laundry products and high temperature water for inactivating MPXV on fabrics. Cotton and polyester fabrics were inoculated with MPXV for 1 minute, placed in a microcentrifuge tube containing water or water with test product for 20 minutes, with agitation every 5 minutes to simulate manual washing.

Results. Sodium hypochlorite, liquid sanitizer, and 2 powdered laundry detergents dissolved in room temperature water, as well as 70°C water alone, completely inactivated MPXV (>3 $\log_{10}$ reduction or >99.9% inactivation) on both cotton and polyester fabrics.

Conclusions. Given the expected concentrations of MPXV on fabrics, the low transfer rate of viruses from porous surfaces to skin, the effective inactivation of laundry processes, and the expected doses required for infection, we expect the risk of transmission after laundering contaminated fabrics to be low. This study provides evidence to support WHO guidance for MPXV inactivation, reducing the viral load on fabrics to prevent the spread of mpox in both health care and household settings.

Keywords. mpox; orthopoxvirus; fabric disinfection; laundry; health care-associated infection.

Mpox (formerly monkeypox) is a disease caused by the *Mpox virus* (MPXV) that belongs to the Poxviridae family and the *Orthopoxvirus* genus. Poxviridae is a family of large, enveloped, double-stranded DNA viruses, which includes variola, cowpox, vaccinia, and other viruses. Mpox was first identified in humans in the 1970s in Central Africa, and it has been mainly confined to tropical rainforest areas. However, following an outbreak in Nigeria that began in 2017, in May 2022 mpox emerged as a global threat, spreading to many nonendemic countries with over 100 000 cases ultimately reported by 123 countries [1]. There are 2 distinct clades of MPXV, clade I and clade II. Clade I was previously termed the Central African (Congo Basin) clade, and clade II was previously termed the West African clade. Clade II is composed of 2 subclades, IIa and IIb. Clade IIb was responsible for the global outbreak. At the time of writing, new outbreaks of mpox due to clade I MPXV in the Democratic Republic of the Congo are also emerging and causing concern [2, 3].

Studies have shown that MPXV is transmitted to humans through close contact with an infected person or animal, or with contaminated material. Human-to-human transmission can occur through direct physical contact with infectious lesions of the skin or mucous membranes, or bodily fluids from those lesions and respiratory droplets, including face-to-face, skin-to-skin, mouth-to-mouth, or mouth-to-skin contact. Cases of health care-associated infection as well as transmission within a tattoo parlor have also been reported in the United Kingdom, United States, Brazil, and Spain due

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to sharp injuries or contact with fomites, such as contaminated objects or surfaces [4–7]. Transmission through contact with fomites has also been reported elsewhere [8–10]. Although fomite-mediated transmission is not the primary transmission route in endemic and nonendemic countries, there have been documented cases where fomites have been suggested as the most likely transmission route [11, 12].

Transmission within households has been described in several studies [13–15], with risk factors including sharing the same room or bed and using the same crockery as the patient with mpox [16]. Current evidence indicates significant environmental contamination of surfaces and fabrics found in homes and rooms of patients with mpox [17–19]. Significantly, infectious (viable) viruses have been grown from samples collected from surfaces days after the patient had left the premises [20, 21], which underscores the crucial importance of thorough cleaning and disinfection procedures [7]. In one study, scientists found MPXV in a residence 3 days after the patient had departed the house [20]. Samples were collected from diverse areas within the household and included samples taken from nonporous surfaces like electronics and door handles, as well as fabrics such as bedding and towels. Out of the 42 samples gathered, 88% tested positive for MPXV DNA. Notably, the fabric samples exhibited the lowest cycle threshold (Ct) values, potentially indicating a higher concentration of viral DNA [20]. Comparable results were documented in another study [21], where infectious virus was detected 15 days after the patient with mpox had left the house. Samples with lower Ct values were often recovered from fabrics, and the authors were able to quantify infectious virus in 1 of the samples coming from an underwear, determining the presence of 320 infectious virus particles. Consequently, engaging in activities that involve interaction with potentially contaminated fabrics, such as changing the bedding of patients with mpox or sharing beds or clothing, has been identified as a potentially risky undertaking [17–19].

The declaration of mpox as a public health emergency of international concern highlights the need for interventions to interrupt virus transmission, including transmission via fabrics. To prevent indirect transmission of MPXV (ie, through fomites such as clothing), the World Health Organization (WHO) advises washing clothes using regular laundry detergent preferably using water at 60°C–90°C or as an alternative soaking the clothes in chlorine, based on the general consensus of virus inactivation [22–24]. Due to the lack of available research with MPXV, there is uncertainty about the precise concentration of chlorine or the amount of risk reduction that might be achieved. Nevertheless, there is general consensus based on evidence from other viruses that the addition of chlorine is likely to reduce residual contamination and this may be particularly useful where thermal disinfection, dilution, and mechanical action is suboptimal. Several factors influence the survival and inactivation of viruses on clothes, such as the type and concentration of the virus, the type and amount of fabric [25, 26], environmental conditions, and laundering parameters [27, 28]. In health care settings, where the risk of exposure to pathogenic viruses is high, laundry protocols are designed to achieve a high level of disinfection using elevated temperatures, complex procedures, and chemical additives [29, 30]. However, these protocols may not be feasible or desirable in domestic settings, and alternative methods to reduce viral load on clothes are needed. Therefore, we aim to investigate the efficacy of manual washing in removing or inactivating viruses from clothes. Specifically, we evaluated the efficacy of different laundry products and cleaning agents as well as the use of high temperature for inactivating MPXV on fabrics. This study provides evidence to support the development of guidelines for MPXV inactivation on linens to prevent the spread of mpox in health care and household settings.

METHODS

MPXV Propagation

An MPXV isolate (isolate 2225/22 Slovenia ex Gran Canaria, clade IIb) was amplified using BHK-21 cells (Syrian Golden Hamster) as described previously [31]. BHK-21 cells were maintained at 37°C with 5% CO_2 in Eagle's Minimum Essential Medium (EMEM; Corning) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich) and 0.05 mg/mL gentamicin (Gibco). For infection, the BHK-21 cells were cultured in EMEM media supplemented with 2% FBS. To amplify MPXV, a T-150 flask of confluent BHK-21 cells was inoculated with 20 µL of a 10^6 plaque-forming unit (PFU)/mL stock of MPXV and incubated for 4 days. Subsequently, the media was recovered and centrifuged for 15 minutes at 5000 rpm to remove any remaining cells and cell debris. Finally, the MPXV stock solution at a concentration of approximately 10^7 PFU/mL was aliquoted and stored at −80°C until use.

MPXV Enumeration

Standard plaque assays were employed to enumerate infectious MPXV using Vero E6 cells (African green monkey kidney cells; Public Health England) as described previously [31]. Vero cells were maintained at 37°C with 5% CO_2 in Dulbecco's Modified Eagle's Medium (DMEM; Corning), supplemented with 10% FBS and 0.05 mg/mL gentamicin. The standard plaque assay was performed as follows: samples were serially diluted and inoculated on a confluent monolayer of Vero E6 cells. One hour after infection, an overlay of 0.8% Methyl Cellulose (Sigma-Aldrich) and DMEM media supplemented with 2% FBS was applied to the cell monolayer and incubated at 37°C with 5% CO_2 for 5 days. Following incubation, the cells were fixed with formalin 10% (VWR International) and stained with crystal violet (Sigma-Aldrich) before quantifying plaques.

All experiments were conducted in Containment level 3 laboratories at The Liverpool School of Tropical Medicine by personnel (A. K. P., S. R.) trained in the relevant codes of practice and standard operating procedures and fully vaccinated against MPXV (2 doses of Imvanex vaccine).

Laundry Formulations

We selected 5 different test products or treatments commonly used for laundry in low-resource settings, including 0.05% sodium hypochlorite solution (NaClO, Reagecon), Dettol Laundry Sanitizer (1.44% dicapryl/dicaprylyl dimonium chloride and 0.96% benzalkonium chloride) 22 mL/L, Ariel handwashing powder 3.75 g/L (Nigeria), OMO washing powder 3.75 g/L (Nigeria), as well as water at 70°C. Additionally, we evaluated water at room temperature and a no-wash control. Detergents were prepared according to the manufacturer's instructions. The solution of sodium hypochlorite 0.05% (500 ppm) was prepared on the day of the experiment by diluting 5% w/v NaClO at a ratio of 1:100. The concentration was measured before each experiment using chlorine test strips according to the manufacturer's instructions (Serim Monitor for Chlorine 100–750 ppm); these have been validated previously for measuring chlorine in water [32].

Water Characteristics

All experiments were conducted using deionized water. The water used for the experiments had a pH of 7 and a total hardness of 0 ppm (soft water). The pH and hardness were measured before the experiments using Serim Monitor strips for pH 0–14, and Serim Monitor for Water Hardness strips, respectively. All experiments were conducted at room temperature, ranging between 21°C and 22°C, with a humidity level of 30%–50%.

Laundry Methodology

A washing methodology was designed to replicate, in the laboratory, manual washing procedures commonly practiced in low-resource settings (Figure 1). Briefly, we inoculated 1-cm² squares of presterilized white cotton (100%) or polyester (100%) fabric with 10 µL of MPXV (approximately 10^7 PFU/mL) suspended in media simulating dirty or clean conditions. Dirty conditions were prepared by mixing 9 volumes of MPXV viral stock with 1 volume of interfering substance, bovine serum albumin (BSA; Fisher Scientific) 3 g/L, while clean conditions were prepared by mixing 9 volumes of viral stock with 1 volume of phosphate-buffered saline (PBS; Gibco). After virus inoculation, we allowed the contaminated fabric to absorb the inoculum for 1 minute. Subsequently, the fabric was placed in a microcentrifuge tube containing 1.5 mL of water with test product or water alone for 20 minutes. Throughout the 20 minutes, the fabric was stirred every 5 minutes with an inoculation loop, simulating the physical movements involved in washing clothes. For the heat treatment method, the washing step was performed using water at 70°C by placing the microcentrifuge tube containing sterile deionized water in a heat block. After washing, the fabric was rinsed with sterile deionized water by placing the fabric in a microcentrifuge tube with 1.5 mL of water, moving the fabric in and out of the tube 5 consecutive times. Finally, the fabric was removed using sterile tweezers and placed in a collection tube containing 1 mL of ice-cold recovery media (DMEM + 2% FBS) for neutralization and subsequent quantification. The tubes were

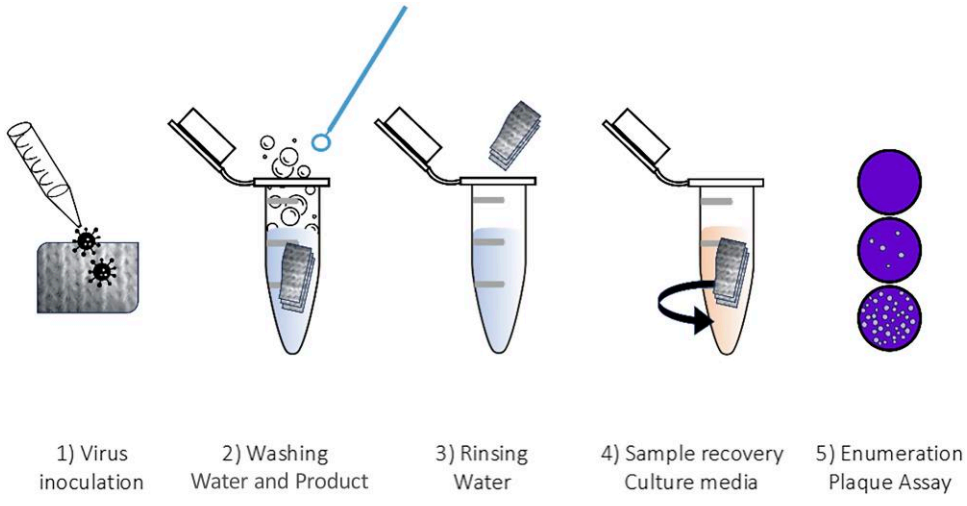

Figure 1. Washing method: (1) inoculate 10 µL of mpox virus at a concentration of 10^7 plaque-forming units/mL onto the fabric and allow the inoculum to absorb for 1 minute; (2) place the fabric in a tube containing 1.5 mL of the test product for 20 minutes, stirring using an inoculation loop every 5 minutes; (3) rinse the fabric in water; (4) place the fabric in the recovery tube containing culture medium and vortex it to recover the virus on the fabric; and (5) quantify the sample using a plaque assay.

Table 1. Efficacy of Laundry Products in Inactivating Mpox Virus on Fabric

	Polyester				Cotton			
	Clean Condition		Dirty Condition		Clean Condition		Dirty Condition	
Treatment	Concentration, $\log_{10}$ PFU	LRV	Concentration, $\log_{10}$ PFU	LRV	Concentration, $\log_{10}$ PFU	LRV	Concentration, $\log_{10}$ PFU	LRV
Virus titer	4.5 ± 0.1	…	4.6 ± 0.2	…	4.0 ± 0.0	…	4.2 ± 0.1	…
Water at 22°C	3.3 ± 0.1	1.2	2.8 ± 0.2	1.9	2.6 ± 0.1	1.3	2.7 ± 0.0	1.5
Water at 70°C	≤1.0 ± 0.0	≥3.5	≤1.0 ± 0.0	≥3.6	≤1.0 ± 0.0	≥3.0	≤1.0 ± 0.0	≥3.2
Sodium hypochlorite 0.05%	≤1.0 ± 0.0	≥3.5	≤1.0 ± 0.0	≥3.6	≤1.0 ± 0.0	≥3.0	≤1.0 ± 0.0	≥3.2
Liquid disinfectant	≤1.0 ± 0.0	≥3.5	≤1.0 ± 0.0	≥3.6	≤1.0 ± 0.0	≥3.0	≤1.0 ± 0.0	≥3.2
Powder detergent 1	≤1.0 ± 0.0	≥3.5	≤1.0 ± 0.0	≥3.6	≤1.0 ± 0.0	≥3.0	≤1.0 ± 0.0	≥3.2
Powder detergent 2	≤1.0 ± 0.0	≥3.5	≤1.0 ± 0.0	≥3.6	≤1.0 ± 0.0	≥3.0	≤1.0 ± 0.0	≥3.2

Data shows mean values (± SD) of 3 independent replicates. The limit of detection of the assay was 10 PFU/mL (1 $\log_{10}$ PFU/mL).

Abbreviations: LRV, log reduction value; PFU, plaque-forming unit.

The data presents the average $\log_{10}$ virus concentration and LRV of mpox virus in fabric (100% cotton and 100% polyester) with and without the washing intervention. Two conditions were assessed for each fabric: dirty (bovine serum albumin-containing media) and clean (phosphate-buffered saline-containing media). The evaluated conditions included 2 powder detergents (Ariel and OMO), a liquid sanitizer (Dettol), a 0.05% sodium hypochlorite solution, and water at room temperature and at 70°C.

vortexed for 5 seconds before removing the fabric from the tube. Samples were quantified using standard plaque assays as previously described. A water control and an untreated control were run alongside each experiment. A product neutralization and cytotoxicity assessment was also conducted as described in the Supplementary Material. Reduction of virus infectivity was calculated from differences in the logarithmic virus titer before (untreated control) and after treatment. Temperature and humidity were monitored throughout the duration of the experiment.

RESULTS

Fabric Disinfection

We assessed the efficacy of various laundry treatments, including water at room temperature, water at 70°C, 0.05% sodium hypochlorite solution, a liquid laundry sanitizer, and 2 powder detergents commonly used in mpox-endemic regions. Our data reveals that, in the absence of detergents and cleansing solutions, washing fabrics with water at room temperature for 20 minutes resulted in a 1.2–1.9 $\log_{10}$ reduction of MPXV in the fabrics (Table 1). Furthermore, there was a ≥ 3 $\log_{10}$ (≥99.9%) reduction in MPXV on cotton and polyester by including thermal inactivation during the washing process using water at 70°C. This aligns with published literature demonstrating that MPXV suspended in various liquid matrices is successfully inactivated (>4 $\log_{10}$ reduction) in less than 5 minutes at 70°C and less than 15 minutes at 60°C [33].

The present study demonstrates the effectiveness of commonly used laundry products in inactivating MPXV (>3 $\log_{10}$ reduction or >99.9% inactivation) in both cotton and polyester fabrics (Table 1 and Figure 2). Specifically, the 2 laundry powders assessed, along with the liquid cleansing solution, completely inactivated the MPXV on the fabric samples, whether in the presence or absence of interfering substance (dirty condition, 3 g/L of BSA). Additionally, the use of a 0.05% chlorine solution resulted in complete inactivation of the virus in both cotton and polyester fabrics, under both clean and dirty conditions (Table 1 and Figure 2).

DISCUSSION

To assess if laundry practices are adequate to reduce the risk of infection below an acceptable threshold, we must have information not only on the efficacy of washing, but also on the level of contamination expected on fabrics, the persistence of MPXV on fabrics, the efficacy of laundry processes, the transfer efficiency of MPXV from fabrics to people, and the dose-response relationship for causing infection. Limited data exist regarding the expected levels of infectious MPXV contamination of fabrics and the persistence of the virus on fabrics over extended periods of time. However, there is evidence of substantial contamination in the homes and hospital rooms of patients with mpox and the DNA of MPXV has been detected on a variety of fabrics [18, 20, 21, 34]. Atkinson et al (2022) detected elevated levels of MPXV DNA (Ct values of 22–31) on mattress sheets, towels, and bedding in the home of a patient with mpox 3 days after the patient had left. Furthermore, live virus was isolated from 2 of the fabric samples tested [20]. Similar results were reported by Nörz et al (2022) who detected between 10^2 and 10^5 DNA copies/cm^2 on fabrics, with successful virus isolation from 1 sample [18]. However, these studies did not attempt to quantify infectious virus. To our knowledge, only 1 study, Morgan et al (2022), attempted the quantification of infectious virus on fabrics in addition to DNA quantification [21]. They were able to detect MPXV DNA on towels, underwear, and bedding and recovered 3.2×10^2 PFU from 1 underwear sample but were unable to quantify virus on the other

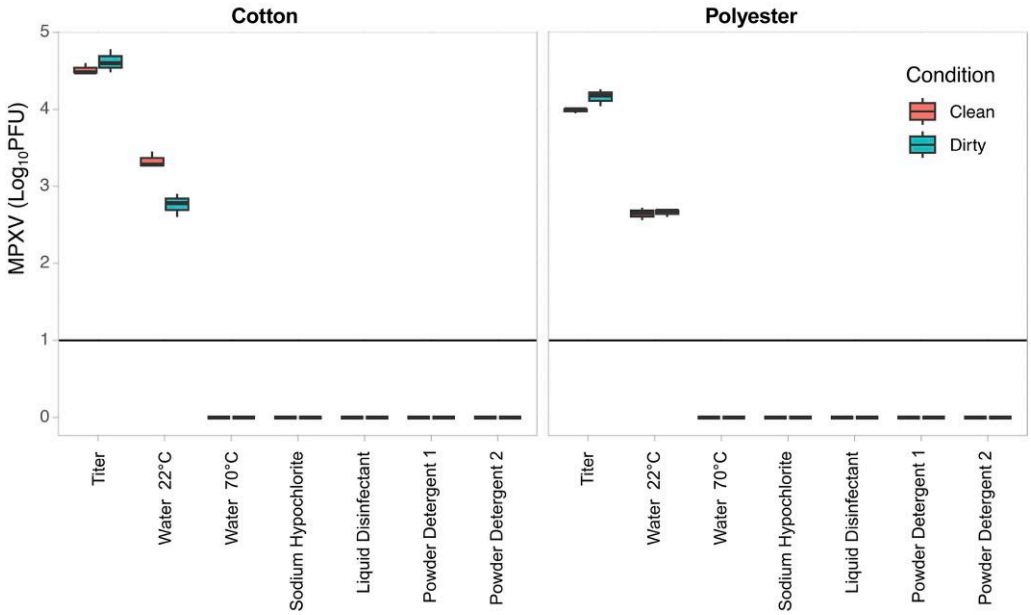

Figure 2. Efficacy of laundry products and methods inactivating mpox virus (MPXV) from fabric. The graph illustrates the quantity of MPXV in $\log_{10}$ plaque forming units (PFU) on the fabric before (titer) and after washing fabrics for 20 minutes using various detergents, sodium hypochlorite 0.05%, and water. The experiment involved fabrics of 100% cotton and 100% polyester. MPXV was inoculated on the fabrics in a solution of media and phosphate-buffered saline (clean condition) or media and bovine serum albumin (dirty condition). All experimental conditions were run at room temperature (22°C), except for water at 70°C. The boxplots summarize triplicate data, representing the mean (line), first and third quartile (box) and maximum/minimum (whiskers). The limit of detection was 10 PFU. Values below the limit of detection are presented as 0.

samples. Notably, these samples were collected from the home of a patient with mpox 15 days after the patient had vacated the place [21]. Therefore, we can expect that some fabrics in contact with patients with mpox may contain levels as high or higher than the 3.2×10^2 PFU previously reported.

In this study we evaluated the efficacy of laundry detergents and other cleaning methods using a protocol designed to mimic laundry processes with manual washing, as it is more representative of common laundry practices in endemic countries [35, 36]. We evaluated 2 powder detergents, a liquid sanitizer, a low-concentration chlorine solution, and water at 70°C, as possible treatments. We found that all treatments successfully inactivated MPXV with or without soiling. The soiled condition, representing dirty clothes or those with organic matter, contained high protein concentrations. Due to the experimental limit of detection and the initial inoculum concentration, we observed a log reduction $\geq 3 \log_{10}$. Therefore, we expect to observe at least a 3-$\log_{10}$ reduction while washing using laundry products following manufacturer's instructions for a period equal to or longer than 20 minutes.

Another important component when evaluating risks and assessing risk reduction strategies is the calculation of the expected dose that the susceptible individual may receive [37]. For example, if we assume that there could be fabrics contaminated with concentrations of MPXV as high as 10^4 PFU (2 orders of magnitude higher concentration than the highest observed), the laundry processes described herein will reduce that concentration more than 3 $\log_{10}$, to a final concentration $\leq$10 viruses; however, this does not imply that all these viruses will end up in the susceptible individual. The number of viruses transferred will depend on the activity performed and the environment. For example, it was speculated that changing bedding of a patient with mpox could transfer viruses through fomite-to-skin contact or through inhalation of virus particles while carrying bedlinen, particularly in the absence of facial mask use by the health worker [7, 12]. The transfer efficiency of viruses from porous materials, such as fabrics, has been shown to be significantly lower than that of nonporous materials [38], fluctuating between 0% and 2.6% [38, 39], depending on the virus, the material, and environmental conditions. Therefore, it is reasonable to expect that less than 10% of the viruses would be transferred to the susceptible individual upon skin contact with contaminated fabrics.

Finally, it is important to acknowledge that viruses exhibit varying infectious doses, a factor of significance when evaluating the risks associated with human interaction with contaminated environments. Numerous studies offer insights into MPXV infection using in vivo models. Animals commonly employed in studying MPXV infection include mice, rabbits, prairie dogs, primates, rats, and squirrels [40, 41]. An ideal animal model should exhibit a viral infection pattern comparable to that seen in humans. Despite inherent limitations, animal

models provide valuable information when investigating infectious dose. Most animal studies aim at understanding MPXV infection; therefore, the animals are often inoculated with high doses of virus (10^4–10^6 PFU) to observe infection after inoculation and there are limited data on infectivity using low doses (1–10^3 PFU) [40, 41]. Studies infecting prairie dogs intranasally showed that a dose of 6×10^2 PFU led to a 25% infection rate (1 out of 4 prairie dogs were infected), as compared with 100% infection rate (4 out of 4 infected) when the dose was 10 times higher [42, 43]. Small animal models are usually considered to be conservative, meaning that we expect the infectious dose to be higher for humans than for small animals. Therefore, it is likely that infectious dose for humans will be higher than 10^2 PFU and will depend on many factors including the inoculation route and MPXV strain [40, 41].

The study presented certain limitations, which warrant consideration. The concentration of virus inoculated on the fabric stood at approximately 10^7 PFU/mL. While this concentration, coupled with the methodology employed, enabled the observation of a log reduction ≥ 3 $\log_{10}$, higher reduction values remained unattainable due to the limit of detection of the assay employed. Our evaluation was also restricted to a single washing time of 20 minutes. This duration was chosen to simulate a worst-case scenario, acknowledging that typical laundry processes extend beyond this timeframe. However, it is worth noting that some individuals may opt for shorter washing periods, a scenario not specifically addressed within this study. Further studies are needed to explore the relationships between washing time and viral inactivation, as washing times can vary significantly. It is also important to note that the study does not investigate mechanical washing using washing machines. Washing machines are expected to be more effective at inactivating MPXV from fabrics as they incorporate heat inactivation along with the microbiological activity of detergents, coupled with increased contact time between the product and the virus, which is expected to be more effective at reducing virus load. Additionally, although we designed the method to maintain the concentration of the product recommended by manufacturers for hand-washing (ratio of water to product), our study was conducted at small scale, which does not reflect the volumes of water and product expected to be present in real-world hand-washing procedures.

Additionally, our investigation relied solely on a single concentration of BSA (3 g/L) as a surrogate for soil conditions. Differing soil compositions could conceivably yield divergent outcomes. Furthermore, we evaluated a limited number of commercially available products as well as high temperature water (70°C) and a low concentration of sodium hypochlorite (0.05%). The use of laundry-based decontamination strategies may not be feasible for vulnerable populations currently facing mpox such as displaced populations, therefore, alternative decontamination strategies should be further explored. Lastly, it is important to note that these experiments were conducted only on MPXV clade IIb; however, we surmise that the results may be applicable to clade I from a disinfection mechanisms standpoint.

Although more data are needed to know for certain that current laundry practices are effective at eliminating transmission risks, it is reasonable to think they will reduce it significantly, making the risk of transmission through fabrics after laundering very unlikely. In our study, we showed ≥ 3 $\log_{10}$ reduction of MPXV using commercially available powder detergents and a liquid sanitizer, as well as low concentration of chlorine solution and water at high temperatures. Given the expected concentrations of MPXV on fabrics, the low transfer rate of viruses from porous surfaces to skin, the effective reduction of mpox through laundry processes, and the expectation that doses required for infection are higher than 10^2 PFU, we expect the risk of transmission after laundering of contaminated fabrics to be low. Furthermore, it is anticipated that a laundry process incorporating heat inactivation along with the microbiological activity of detergents, coupled with increased contact time between the product and the virus, will enhance the effectiveness of the laundry process. Therefore, laundry processes in washing machines are expected to be highly effective at significantly reducing MPXV from fabrics.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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