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Original Research

Vaccinia virus viability under different environmental conditions and different disinfectants treatment

Shan Mei [a,1], Liang Wei [a,1], Yu Xie [a,1], Fei Zhao [a], Yu Huang [a], Zhangling Fan [a], Yamei Hu [a], Liming Wang [a], Lingwa Wang [a,b], Ying Wang [c,*], Fengwen Xu [a,*], Fei Guo [a,*]

[a] NHC Key Laboratory of Systems Biology of Pathogens, National Institute of Pathogen Biology and Center for AIDS Research, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100730, China
[b] Department of Otolaryngology Head and Neck Surgery, Beijing Tongren Hospital, Capital Medical University, Beijing 100730, China
[c] Clinical Laboratory, Emergency General Hospital, Beijing 100028, China

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ABSTRACT

Monkeypox (mpox) outbreak in 2022 has caused more than 91,000 cases, has spread to 115 countries, regions, and territories, and has thus attracted much attention. The stability of poxvirus particles in the environment is recognized as an important factor in determining their transmission. However, few studies have investigated the persistence of poxviruses on material surfaces under various environmental conditions, and their sensitivity to biocides. Here, we systematically measured the stability of vaccinia virus (VACV) under different environmental conditions and sensitivity to inactivation methods via plaque assay, quantitative real-time polymerase chain reaction (qPCR), and Gaussia luciferase (G-luciferase) reporter system. The results show that VACV is stable on the surface of stainless steel, glass, clothing, plastic, towel, A4 paper, and tissue and persists much longer at 4 °C and −20 °C, but is effectively inactivated by ultraviolet (UV) irradiation, heat treatment, and chemical reagents. Our study raises the awareness of long persistence of poxviruses in the environment and provides a simple solution to inactivate poxviruses using common disinfectants, which is expected to help the control and prevention of mpox virus and future poxvirus outbreaks.

1. Introduction

As of September 30, 2023, over 91,123 mpox (also known as monkeypox) cases have been confirmed in 115 countries, regions and territories (https://worldhealthorg.shinyapps.io/mpx_global/). An outbreak of mpox in the Chinese mainland in 2023 was reported, with a total of 1,403 new confirmed cases of mpox from June 2 to September 30, 2023 (https://www.chinacdc.cn/jkzt/crb/zl/szkb_13037/). Mpox is a zoonotic disease caused by monkeypox virus which was first isolated in 1958 from an imported cynomolgus macaque in Denmark; human cases were first reported in the early 1970s in central Africa [1–3]. After the eradication of smallpox in 1980 and the cessation of smallpox vaccination worldwide, mpox virus infection has emerged as a major orthopoxvirus of public health concern [4].

Mpox virus is a member of the genus *Orthopoxvirus* in the family *Poxviridae*, which also includes variola virus, vaccinia virus (VACV), camelpox virus, cowpox virus, and others, and is the largest known enveloped double-stranded DNA virus. Their genomes vary in size from 140 kbp of parapox virus to 300 kbp of fowlpox virus and can encode more than 200 genes [5]. Poxviruses, different from other DNA viruses, replicate in the cytoplasm and do not depend on host DNA and RNA replication machinery because they encode their own DNA polymerase, DNA-dependent RNA polymerase, transcription factors, capping enzyme, and methylases [6]. Vaccinia virus has been used as a vaccine for mass vaccination against smallpox in the 19th and 20th centuries and also as a surrogate for variola virus research [7]. As a result of their high conservation, VACV and other poxviruses show similarities in their stability in human excretions and on common surfaces and are highly resistant to drying [8–11]. Therefore, effective disinfection methods of the environment and contaminated objects are essential to prevent poxvirus transmission. However, few systematic investigations have been conducted to examine the stability of mpox virus and related poxviruses on different types of materials and under various environmental conditions. The effectiveness of commonly used disinfection methods in inactivating poxviruses has also been inadequate for these viruses.

Here, we investigated the persistence of the VACV as an example of poxviruses on the eight common materials, in three mediums, at differ-

* Corresponding authors: NHC Key Laboratory of Systems Biology of Pathogens, National Institute of Pathogen Biology and Center for AIDS Research, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100730, China (F. Xu and F. Guo); Clinical Laboratory, Emergency General Hospital, Beijing 100028, China (Y. Wang).
E-mail addresses: shidianpeng@sohu.com (Y. Wang), xanadufw@aliyun.com (F. Xu), guofei@ipb.pumc.edu.cn (F. Guo).
[1] These authors contributed equally to this work.

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HIGHLIGHTS

Scientific question

The outbreak of mpox (also known as monkeypox) in multiple regions worldwide since 2022 has elicited public concerns. Nonetheless, there is a dearth of comprehensive research of poxvirus regarding its survivability in various environments and its susceptibility to disinfectants.

Evidence before this study

The mpox virus has the ability to infect various species of mammals, and it is believed that terrestrial and arboreal rodents are capable of transmitting mpox to humans. Transmission occurs primarily through direct contact with body fluids or lesions, respiratory droplets, and contaminated objects, and there have been reports of sustained human-to-human transmission during this epidemic. Vaccinia virus and other poxviruses exhibit similarities in their stability in human excretions and on commonly touched surfaces due to their high conservation. Therefore, it is imperative to understand the persistence of vaccinia virus in the environment and its susceptibility to various disinfection methods.

New findings

In this study, we conducted a systematic investigation to determine the stability of the vaccinia virus, which serves as a representative of poxviruses, under various environmental conditions and assess its susceptibility to inactivation methods using plaque assay, quantitative real-time polymerase chain reaction (qPCR), and the Gaussia luciferase (G-luciferase) reporter system. Our findings provide compelling evidence that the vaccinia virus remains stable on majority of tested materials and media. Furthermore, we observed that the virus remains viable for prolonged periods at temperatures of 4 ˚C and 20 ˚C, while it can be effectively inactivated through ultraviolet (UV) irradiation, heat treatment, and chemical agents.

Significance of the study

Our study emphasizes the prolonged viability of poxviruses in the environment and presents a simple yet effective approach to inactivate them using easily accessible disinfectants. This research is expected to contribute to the control of mpox virus and prevention of future poxvirus outbreaks.

ent temperatures and upon exposure to five common disinfection methods. Using plaque assay, Gaussia luciferase (G-luciferase) reporter system, and quantitative real-time polymerase chain reaction (qPCR) to quantify the infectious virus particles and viral DNA, we demonstrated that VACV is stable on the surfaces of most materials and mediums, and is effectively inactivated by multiple viral inactivation methods including ultraviolet (UV) irradiation, heat, and chemical reagents. These data inform effective practice in environmental decontamination and transmission control of poxviruses.

2. Materials and methods

2.1. Cell culture

HEK293T (Human, kidney) and Vero (African green monkey, kidney) cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10 % fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (0.1 mg/mL). All cells were incubated at 37 ˚C and 5 % CO_2.

2.2. Propagation of VACV

The vaccinia virus (TianTan Strain) was obtained from National Institute for Viral Disease Control and Prevention, China CDC. Flask 75 (Thermo Fisher Scientific) of Vero cells were infected with VACV at 0.1 multiplicity of infection (MOI) in 2 mL dulbecco's modified eagle medium (DMEM) (HyClone™). Flasks were incubated for 2 h at 37 ˚C and 5 % CO_2. After 2 h, 10 mL of DMEM's medium supplemented with 2 % FBS was added to flasks for 2 to 3 days until optimal cytopathic effects (CPE) were observed. Freeze and thaw the cell and suspension three times by placing it alternately at −80 ˚C and room temperature. Remove cell debris by centrifugation at 1,000 g for 10 min and collect the supernatant. Supernatants were then stored at −80 ˚C.

2.3. Test method

Eight material types and three media were dropwise added VACV using pipettes and exposed to three environmental conditions (three temperatures and the same humidity) for different periods. For each material in each experiment, three replicate test groups, one positive control, and one negative control, were used. Test materials were sterilized before testing via autoclave. Each material was inoculated with 4.25×10^5 plaque forming unit (PFU) virus which was chosen to adequately assess persistence and disinfection efficacy. After an elapsed time ranging from 1 to 28 days, the viruses were extracted from each group and quantified as described [10]. Briefly, the virus was extracted from the individual test materials by placing them into a sterile 1.5 mL eppendorf (EP) tube vial containing 1 mL of phosphate buffered saline (PBS). The tubes were then agitated at 200 rpm/min on an orbital shaker for 15 min at room temperature and then stored at −80 ˚C until further analysis.

2.4. Inactivation method

2×10^5 PFU VACV (10 μL) was added to the tubes and placed in UV irradiation (under 30 W UV lamp) for 30 min, 60 min, and 90 min, incubated at 56 ˚C for 30 min, or at 60 ˚C for 10 min. The following common decontamination chemicals were tested: 75 % ethanol, 0.5 % NaOCl, and 3 % H_2O_2. These chemicals were mixed with viral stocks (volume: volume) at 10:1 (disinfectant: virus) ratio. Incubation times were 0.5 min, 1 min, and 10 min. After treatment, samples were stored at −80 ˚C until further analysis.

2.5. Plaque assay

Vero cells were seeded in 12-well culture plates at 2.5×10^5 cells/well and incubated at 37 ˚C, 5 % CO_2, in saturated humidity. After reaching > 90 % confluence, cells were incubated for 2 h with 0.3 mL virus extract which was serially diluted (10 times) at 37 ˚C, 5 % CO_2. Cells were washed 3 times with PBS. A 1 mL overlay of 1 % agarose solution, penicillin (100 U/mL), streptomycin (0.1 mg/mL), and 2 % FBS was added to the cells and left at room temperature (RT) until the media solidified. Then the plates were incubated at 37 ˚C, 5 % CO_2 for 3 days. Viral plaques were counted on the last day.

2.6. qPCR analysis

Total DNA was isolated from the VACV or the samples using the QIAamp® DNA Mini kit (QIAGEN) according to the manufacturer's protocol. Each DNA sample was subjected to qPCR analysis with specific primers and the Fast SYBR Green Master Mix (Thermo Fisher Scientific). Primers for qPCR were designed with Primer Premier6 software,

and their sequences are as follows: E9 (a protein in VACV), 5′-TGGC AAACCGTAACATACCG-3′ (forward), and 5′-AGGCCATCTATGATTC CATGC-3′ (reverse). qPCR assays were further analyzed by Bio-Rad CFX Manager.

2.7. G-luciferase reporter system

The G-luciferase reporter system was performed as previously described [12]. A G-luciferase reporter plasmid regulated by the VACV p7.5 promoter was constructed. HEK293T cells were cultured in 12-well plates and transfected with the reporter plasmid and then infected with the VACV for 2 h. Cells were washed 3 times with PBS and fresh medium with 2 % FBS were added. Then the plates were incubated at 37 °C, 5 % CO_2 for 24 h or 48 h. Luciferase activity in culture supernatants was measured with Coelenterazine h (Promega, S201A).

2.8. Statistical analysis

Statistical significance was assessed using a *t*-test. All statistical analyses were performed using GraphPad Prism (version 8.0) software.

3. Results

3.1. Persistence of VACV on materials and medium at different temperatures

To investigate the environmental persistence of VACV on materials at different temperatures, we applied VACV (4.25×10^5 PFU) onto the surfaces of eight materials and incubated them at RT (ranging from 23 to 28 °C), 4 °C or −20 °C which simulate the conditions of cold chain transport and refrigeration. Immediate virus recovery (0 day) varied between materials, the PFU values were 2.80×10^5, 2.64×10^5, 3.30×10^4, 2.10×10^5, 3.90×10^5, 2.50×10^5, 1.10×10^5, 1.32×10^5 for stainless steel, glass, wood, clothing, plastic, towel, A4 paper, and tissue, respectively (Supplementary Table 1). The amount of recovered VACV is sufficiently high for monitoring the loss of virus infectivity over time. Virus samples were then collected from the surfaces of tested materials over a 28-day period, and infectious particles were determined in plaque assay. No infectious viruses were recovered from wood from day 7, stainless steel, and glass by day 28 at RT (Fig. 1A, and Supplementary Table 1). Viral titres were reduced by folds of 1.94×10^2, 1.03×10, 7.81×10, 2.78×10^2, and 2.06×10^2 on the clothing, plastic, towel, A4 paper, and tissue on the twenty-eighth day at RT. At 4 °C, the VACV was more stable on all materials tested than at RT, and was only lost by day 28 on wood (Fig. 1B, and Supplementary Table 1). At −20 °C, no significant reduction of virus titre was observed after exposure to all materials for 28 days (Fig. 1B - C, and Supplementary Table 1). These results indicate that the VACV is stable on different materials at low temperatures, especially at −20 °C.

Next, we measured the stability of VACV in PBS, ddH_2O, and maintaining medium (2 % FBS in DMEM) at RT, 4 °C, and −20 °C for 28 days. Virus titres decreased by folds of 1.61×10, 4.13, and 8.99 in PBS, ddH_2O, and 2 % FBS in DMEM, respectively at RT (Fig. 1 D - F, and Supplementary Table 2), by 3.22, 2.67, and 4.26 folds at 4 °C, and by 1.99, 1.84, 1.80 folds at −20 °C by day 28 (Fig. 1D - F, and Supplementary Table 2). The reduction of virus titer in the different media was less than that on the different materials at the same temperature over 28 days. These results suggest that the VACV persists much longer in media, especially at low temperatures.

Real-time PCR is the golden standard for mpox virus detection. We therefore further investigated the persistence of VACV DNA on different materials by qPCR. We tested VACV samples from stainless steel, glass, and PBS after incubation at RT, and samples from wood after exposure at RT, 4 °C and −20 °C. These samples were tested by qPCR because they exhibited more significant reduction over time compared to other samples in virus plaque assays. The trends of reduction in vaccinia viral RNA in these samples corroborated with those of the plaque assays (Table 1). However, viral DNA was still detectable in samples which did not contain infectious VACV as shown by the results of virus plaque assay. This suggests that viral DNA persists when virus infectivity is lost, thus results of PCR test do not warrant the presence of infectious VACV.

3.2. The effects of various inactivation methods on virus viability

We next investigated the effectiveness of UV irradiation, heat treatment, or chemical reagents in inactivating the VACV by virus plaque assays. UV irradiation for 30 min reduced the infectious VACA by 97.5 %, 60 min, and longer UV exposure completely inactivated the VACV (Table 2). Exposure at 56 °C for 30 min or at 60 °C for 10 min was sufficient to inactivate the VACA. Treatment with 75 % ethanol or 0.5 % sodium hypochlorite (NaOCl) for 0.5 min, 1 min, and 10 min resulted in loss of infectious VACV as measured by virus plaque assays. Incubating with 3 % hydrogen peroxide (H_2O_2) for 0.5 min lost half of the infectious VACV, 1 min exposure killed 98.4 % VACA, infectious VACV was not detected after 10 min exposure (Table 2). These results demonstrate that heat treatment, 75 % Ethanol, and 0.5 % NaOCl are more effective in killing the VACV compared to UV irradiation and 3 % H_2O_2.

To further examine the effect of different inactivation methods on the viral genomic DNA, we tested virus samples after exposure to the above disinfection methods by qPCR. UV and heat didn't affect the Ct value levels of viral DNA (Table 2). Viral DNA was readily detectable after treatment with 75 % ethanol and 3 % H_2O_2, the Ct values increased from 1.51 to 3.57 in the 75 % ethanol group and 7.50 to 8.47 in the 3 % H_2O_2 group. However, viral DNA was lost in the 0.5 % NaOCl group (Table 2). These results suggest that 0.5 % NaOCl is the most effective disinfectant in eliminating both infectious VACV and viral DNA.

3.3. Virus survival was detected using the G-luciferase reporter system

Viral plaque assay is time-consuming, PCR test does not inform the presence of infectious viruses. A simple, cost-effective, sensitive, and high-throughput assay is desirable in clinical detection of infectious viruses. Therefore, we developed a G-luciferase reporter assay in which VACV DNA-dependent RNA polymerase stimulates the expression of G-luciferase from viral promoter p7.5 (Fig. 2A)[12]. Live VACVs markedly activated the G-luciferase expression, while viruses inactivated by 56 °C for 30 min did not (Supplementary Fig. 1A). To determine the detection limit of this assay, we tested 1, 10, or 100 PFU of VACVs. As shown in Supplementary Fig. 1B, 1 PFU VACV increased G-luciferase expression by 4-fold 48 h post-infection. G-luciferase activation was more pronounced 48 h after infection than 24 h time point (Supplementary Fig. 1B). These results demonstrate the high sensitivity of G-luciferase reporter assay in detecting VACV infection. Then, we tested virus samples that were exposed to stainless steel, glass and wood using the G-luciferase reporter assay. Exposure to these materials at RT led to loss of infectious VACV as shown by the results of viral plaque assay (Fig. 1A - C). We found that viral samples that were shown positive in viral plaque assay all significantly activated the G-luciferase expression (Fig. 2B and C). Remarkably, significant activation of G-luciferase was observed for virus samples that were negative in viral plaque assay after exposure to for 7 days at RT (Fig. 2C). These results suggest that the G-luciferase reporter assay is more sensitive than the plaque assay in detecting live VACV, is thus more suited in detection of infectious VACV and can be adopted for high-throughput diagnosis.

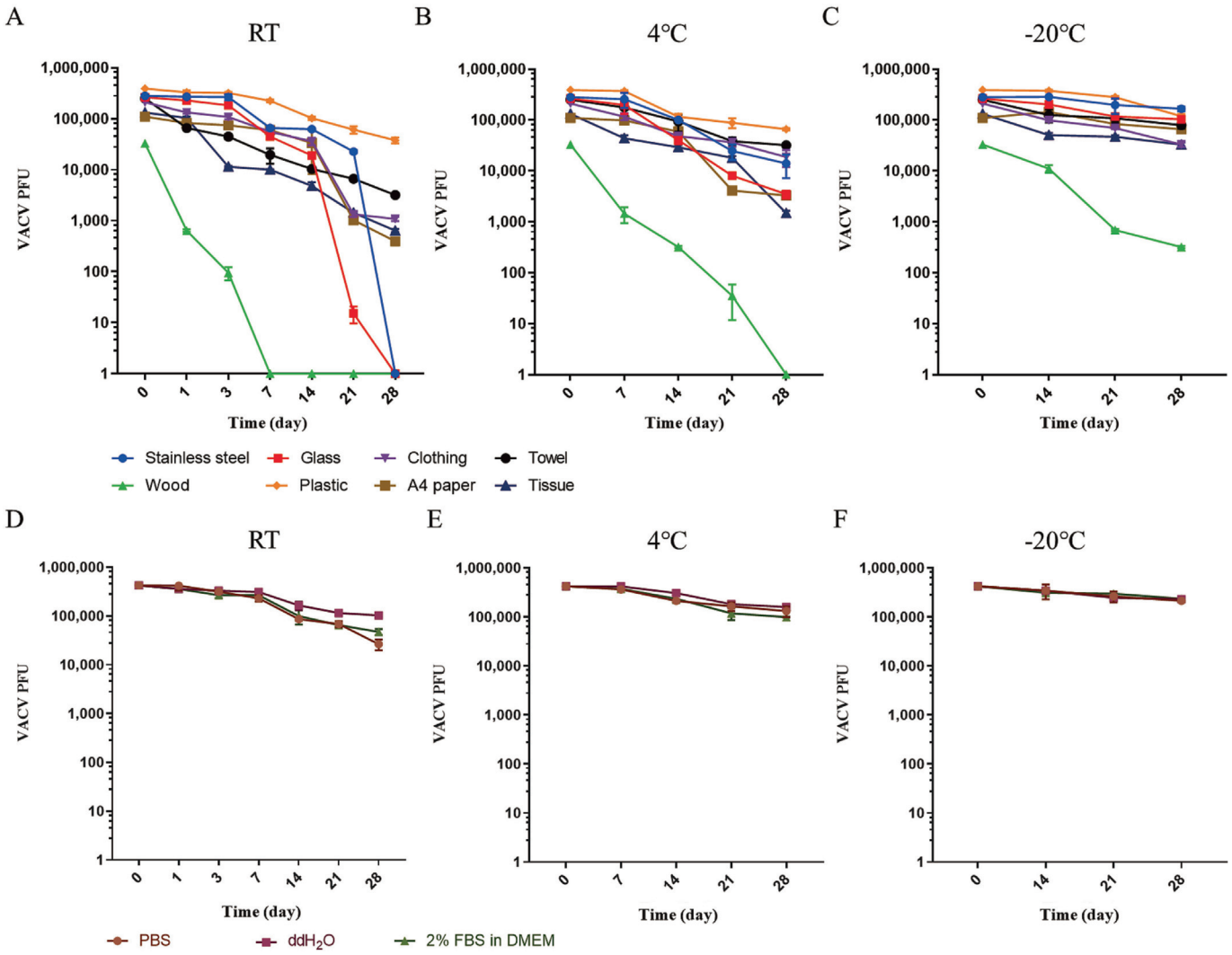

Fig. 1. Persistence of vaccinia virus on surface of different materials or in different media at RT, 4 °C and −20 °C. A) to C) VACAs were applied to the surface of stainless steel, glass, wood, clothing, plastic, towel, A4 paper, and tissue, and maintained at RT, 4 °C, and −20 °C for 0 to 28 days. All samples were quantified by plaque assay. Graphs show the means and standard errors across three replicates. D) to F) VACVs were applied into PBS, ddH_2O, and 2 % FBS in DMEM, and maintained at RT, 4 °C, and −20 °C for 0 to 28 days. All samples were quantified by plaque assay. Graphs show the means and standard errors across three replicates. Abbreviations: VACV, vaccinia virus; PFU, plaque forming unit; RT, room temperature; PBS, phosphate buffered saline; FBS, fetal bovine serum; DMEM, Dulbecco's modified eagle medium.

4. Discussion

Mpox virus is capable of infecting a wide variety of mammals, with terrestrial and arboreal rodents thought to be the source of transmission to humans, while the natural host remains unknown [13]. Transmission is mainly via direct contact with body fluids or lesions, respiratory droplets, and fomites, and sustained human-to-human transmission of the mpox virus during this epidemic has been reported recently [14]. Mpox causes a much milder illness than smallpox with low fatality in humans [15–17]. Different from previous outbreaks with limited cases and transmission range, the rapid global spread and dramatic increase in the number of confirmed cases during mpox outbreak in 2022 raised concerns. Several recent studies showed that the stability of mpox virus is lower than other classic poxviruses. It is crucial to understand the persistence of this virus in the environment and its sensitivity to different disinfection methods.

In this study, we conducted a comprehensive and systematic study of the persistence of poxvirus on multiple materials and sensitivity to various inactivation methods by plaque assay, qPCR, and G-luciferase reporter system, using VACV as a surrogate. Overall, the VACV is remarkably stable on the surfaces of seven common objects and is even more stable in three tested liquid media. The poor viability of VACV on the surface of wood may be a result of several factors, including the low elution efficiency of the wood, the fact that the wood is a flake board, and chemicals such as formaldehyde that may have been added during wood production and can be detrimental to virus survival. In addition, the persistence of the VACV was generally shorter on glass and steel at RT, which is inconsistent with previous research [10]. Low temperatures are associated with better virus survival, particularly at −20 °C. Recent studies have assessed the stability of mpox virus in various environments and evaluated the efficacy of decontamination methodologies [18,19]. These investigations imply that the survival capacity of mpox virus might be inferior to that of other classical poxviruses. However, it is still deemed highly stable, necessitating vigilance regarding the potential risk of transmission, which is in line with our conclusion. Therefore, health and epidemic prevention

Table 1
Quantification of vaccinia virus DNA at specified time points from different materials and media.

Sample	Temperature	Time (day)	PFU	Ct
Stainless steel	RT	1	2.71×10^5	15.54
		7	6.61×10^4	17.84
		21	2.27×10^4	18.26
		28	ND	32.51
Glass	RT	1	2.29×10^5	16.13
		14	1.91×10^3	19.21
		21	1.52×10	25.93
		28	ND	30.76
PBS	RT	1	4.17×10^5	15.14
		14	8.64×10^4	16.05
		21	6.84×10^4	17.48
		28	2.64×10^4	18.97
Wood	RT	1	6.40×10^2	19.59
		3	9.53×10	22.29
		7	ND	30.67
		14	ND	35.70
		21	ND	35.96
		28	ND	37.08
Wood	4 °C	7	1.44×10^3	20.79
		14	3.20×10^2	22.16
		21	3.53×10^0	26.95
		28	ND	37.39
Wood	−20 °C	14	1.11×10^4	18.84
		21	6.92×10^2	22.44
		28	3.20×10^2	23.69

The residual viral DNA from stainless steel, glass, and PBS at RT was determined by qPCR. Abbreviations: RT, room temperature; PFU, plaque forming unit; Ct, threshold cycle of qPCR for virus DNA detection; PBS, phosphate buffered saline; ND, not detected.

Table 2
Results of vaccinia virus inactivation by different methods.

Inactivation Method	Time (min)	PFU	Ct
UV irradiation	30	5.10×10^3	16.37
UV irradiation	60	ND	17.22
UV irradiation	90	ND	17.70
Heat at 56 °C	30	ND	16.81
Heat at 60 °C	10	ND	18.22
75 % Ethanol	0.5	ND	19.81
75 % Ethanol	1	ND	17.84
75 % Ethanol	10	ND	19.90
0.5 % NaOCl	0.5	ND	N/A
0.5 % NaOCl	1	ND	N/A
0.5 % NaOCl	10	ND	N/A
3 % H_2O_2	0.5	1.00×10^5	23.93
3 % H_2O_2	1	3.30×10^3	24.80
3 % H_2O_2	10	ND	23.83
vaccinia virus		2.00×10^5	16.33
NC		ND	N/A

Vaccinia virus was treated by UV irradiation, heat at 56 °C, heat at 60 °C, 75 % ethanol, 0.5 % NaOCl, or 3 % H_2O_2, and then the titer of the remaining virus was measured using plaque assay and viral DNA was analyzed by qPCR. Data are means of three independents experiments. Abbreviations: PFU, plaque forming unit; Ct, threshold cycle of qPCR; UV, ultraviolet; NaOCl, sodium hypochlorite; H_2O_2, hydrogen peroxide; ND, not detected; N/A, not applicable; NC, uninfected group as negative control.

departments and related researchers should pay more attention to the survival of the poxvirus on object surfaces and in low-temperature environments to eliminate the transmission risk.

In addition, we investigated the ability of frequently used methods to inactive VACV. UV treatment for 1.5 h, heat treatment at 56 °C for 30 min and 60 °C for 10 min completely inactivated VACVs. 75 % ethanol and 0.5 % NaOCl for 0.5 min resulted in significant viral inactivation. The ability of 3 % H_2O_2 to inactivate VACV was relatively weaker, requiring about 10 min to completely inactivate the virus. Therefore, appropriate disinfection methods could reduce the risk of VACV infection and protect hospital staff and laboratory researchers. It should be noted that the undiluted samples which contained 75 % ethanol, 0.5 % NaOCl, and 3 % H_2O_2 are cytotoxic to cells. Although some researchers used sodium thiosulfate, as a neutralizer, to combine with NaOCl, it was still toxic to cells in undiluted samples [20]. However, VACV DNA persisted under treatment with UV irradiation, heat, 75 % ethanol, and 3 % H_2O_2. A recent study showed that Zika virus RNA integrity was maintained in all heat-treated samples and at all doses of gamma irradiation with all titers [21]. High amounts of Ebola virus vRNA remains after 70 % EtOH treatment [20]. These data indicate that heat, gamma irradiation, and EtOH may not destroy the viral genome. NaOCl at concentrations of 0.5 % and 1 % significantly reduces but does not completely eliminate vRNA [20]. Therefore, viral nucleic acid detection may not necessarily inform the presence of infectious virus particles in hospitals or other places where multiple disinfection methods are used. Assessment of effective viral inactivation should be based on assays that measure infectious viruses and not only by detection of viral genome.

In addition to using classic plaque assay to detect infectious VACV, we also developed a G-luciferase reporter system with higher sensitivity and greater potential. This system can be used to detect other poxviruses like mpox virus besides VACVs due to the conservation of DNA-dependent RNA polymerase among poxviruses. This reporter assay can also be further optimized, such as constructing G-luciferase cell lines, to increase the consistency across different experiments and improve the system detection capability. In summary, our study demonstrates that poxviruses are stable in the environment and are sensitive to multiple viral inactivation methods. This study advances our understanding of poxvirus persistence and informs the effective control of the epidemic of mpox virus or other poxviruses in the future by preventing virus transmission.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

Author contributions

Shan Mei: Data curation, Investigation, Methodology, Validation, Visualization. **Liang Wei:** Data curation, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Yu Xie:** Data curation, Investigation, Methodology. **Fei Zhao:** Data curation, Investigation, Methodology. **Yu Huang:** Data curation, Investigation. **Zhangling Fan:** Data curation, Investigation. **Yamei Hu:** Data curation. **Liming Wang:** Data curation. **Lingwa Wang:** Data curation. **Ying Wang:** Writing – review & editing. **Fengwen Xu:** Data curation, Investigation, Methodology, Writing – review & editing. **Fei Guo:** Conceptualization, Funding acquisition, Writing – review & editing.

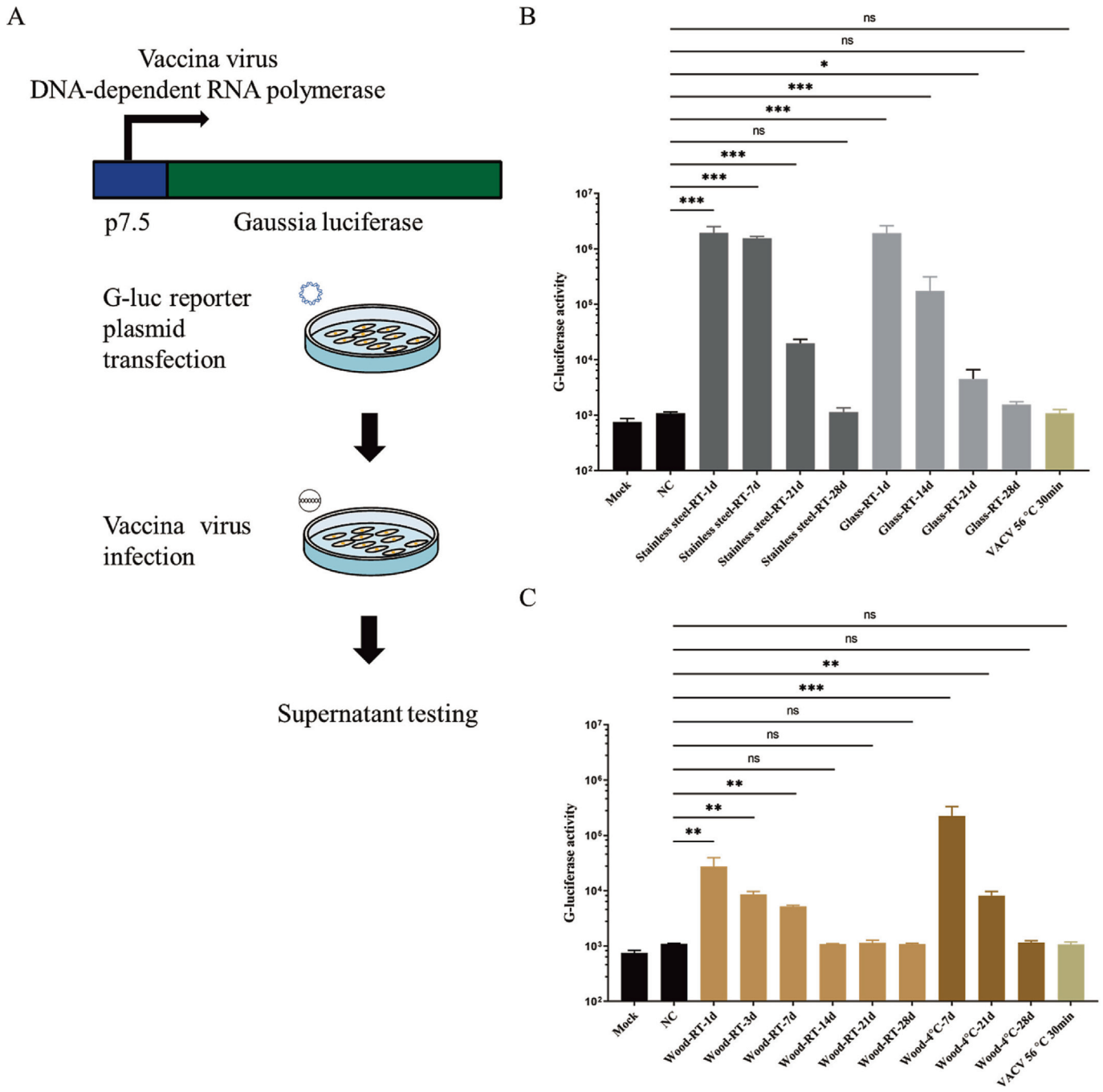

Fig. 2. Persistence of vaccinia virus under different conditions was analyzed by the G-luciferase reporter system. A) Illustration of the G-luciferase reporter system. HEK293T cells were transfected with G-luciferase reporter plasmid and then infected with VACV. Luciferase activity in culture supernatants was determined. B) and C) Samples with different treatments were analyzed by the G-luciferase reporter system at 48 hpi. Graphs show the means and standard errors across three replicates. ns, not significant; $^{*}P < 0.1$; $^{**}P < 0.01$; $^{***}P < 0.001$. Abbreviations: VACV, vaccinia virus; PFU, plaque forming unit; Mock, cells without transfection and infection; NC, the control without infection RT, room temperature; G-luciferase, Gaussia luciferase.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bsheal.2023.12.005.

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