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# Assessing Elimination of Mouse Kidney Parvovirus from Cages by Mechanical Washing

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**Mouse kidney parvovirus (MKPV), a newly identified parvovirus of the genus *Chaphamaparvovirus*, causes inclusion body nephropathy in severely immunocompromised mice and is prevalent in research mouse colonies. As nonenveloped viruses, mammalian parvoviruses are stable and generally resist thermal inactivation; however, as a novel and highly divergent parvovirus, the thermal stability of MKPV is undefined. This study aimed to evaluate the ability of cage sanitization in a mechanical washer to eliminate MKPV. Cages contaminated by MKPV-infected mice were assigned to 1 of 3 treatment groups: 1) control (bedding change only); 2) sanitization in a tunnel washer (88 °C final rinse for 20 s); or 3) sanitization in a tunnel washer followed by autoclave sterilization (121 °C for 20 min). The presence of MKPV on the cage's interior surface was assessed by PCR of cage swab extracts collected before and after cage treatment. After treatment and swabbing, each cage housed 4 MKPV-negative CD1 mice. Each group of naive CD1 mice was assigned to one of the treatment groups and was housed in a cage from this group for two, 1 wk periods. At 12, 17, and 20 wk after the first exposure, renal tissue was collected from 1 test mouse per cage and assessed for MKPV by PCR. MKPV was detected by PCR on the surface of 63% of the pretreatment cages. All cages sanitized in a tunnel washer with or without sterilization were PCR negative after treatment. Seven of 10 mice housed in untreated cages contained a mouse positive for MKPV by 20 wk after exposure. None of the mice housed in cages sanitized in a tunnel washer with or without sterilization tested positive for MKPV at any time point. This study indicates that MKPV contaminated caging can result in MKPV infection of mice, and the use of a tunnel washer at the temperature and duration evaluated was sufficient to remove MKPV nucleic acid and prevent MKPV transmission.**

**Abbreviations:** MKPV, Mouse Kidney Parvovirus; BR, bedding removed only treatment group (control); TW, tunnel wash treatment group; WA, wash then autoclaved treatment group

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## Introduction

The recently discovered viral species *Rodent chaphamaparvovirus 1,* a member of the new parvovirus genus *Chaphamaparvovirus,* is a novel pathogen capable of causing significant clinical disease in severely immunocompromised mouse strains.[12] Mouse kidney parvovirus (MKPV) and murine chaphamaparvovirus (MuCPV) are individual viruses within this species.[11] The prevalence of these viruses in academic mouse colonies is reported to be as high as 9.4% of immunodeficient mice, and 10.9% of immunocompetent mice.[8] MKPV-infected immunocompromised mice develop inclusion body nephropathy which, after a prolonged incubation period, can result in renal insufficiency and eventual mortality.[12] Immunocompetent mice can also become infected and persistently shed virus, although infection remains subclinical and renal pathology is mild.[5] Due to the subclinical nature of infection in immunocompetent mice, the presence of this virus in immunocompetent colonies may go undetected without routine surveillance screening. The ability of this virus to cause significant pathology in immunocompromised mice warrants its consideration as an excluded agent in laboratory animal facilities. Little is known about its infectivity and environmental stability. Understanding the persistence and infectivity of this virus on fomites, including cages, is instrumental in devising strategies to exclude this pathogen from mouse colonies.

Mammalian parvoviruses are unenveloped and, in general, are environmentally stable and resist inactivation by exposure to elevated temperatures, desiccation, and multiple classes of disinfectants. Canine parvovirus (CPV) has been reported to resist inactivation for 1 h at 80 °C and is resistant to many common classes of disinfectants, including ether, chloroform, and alcohol.[9] The closely related feline panleukopenia virus is resistant to all but 3 of 27 disinfectants tested.[14] In addition, the human B19 parvovirus is resistant to ether, chloroform, deoxyribonuclease (DNase), and ribonuclease (RNase) treatment.[10] In a study of minute virus of mice (MVM), a murine parvovirus, substantial inactivation occurred only when virus liberated from mouse fibroblasts was incubated in a water bath at 90 °C for at least 10 min or in NaOH solutions of pH ≥12.8 for 1 min.[2] Furthermore, in a study evaluating thermal resistance of various viruses, 2 parvoviruses, CPV and Kilham rat virus (KRV), were stable at 80 °C for 30 min.[13]

The environmental hardiness of parvoviruses has implications for rodent colony health. Murine parvovirus (MPV) can be transmitted by fomites between cages, although standard cage-wash procedures reduce the level of infectious MPV on cage components to below the threshold needed to initiate an

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infection.[4] Some evidence suggests that unsterilized feed was the source of an outbreak of MPV, despite pelleted food being subjected to temperatures of 65 to 85 °C, pressure, and steam.[15] In addition, another study confirmed that MPV remained infectious after being subjected to conditions reproducing the rodent chow pelleting process.[1]

While genetically distinct from other parvoviruses, MKPV, as a related virus, may have similar thermal resistance. Due to the effects MKPV can have on the health of immunocompromised mouse strains and the possible impact it may have on research carried out on infected animals, an important concern is whether routine cage wash processes are adequate to eliminate the virus on animal caging and prevent transmission. This study aimed to determine whether fomite (cage) transmission of MKPV occurs, and if it does, whether mechanical washing of MKPV-contaminated caging is adequate to prevent fomite transmission to naïve mice or whether the additional step of autoclaving after washing is also necessary.

## Methods

**Experimental design.** ***Pilot study.*** A pilot study was conducted to confirm that MKPV could be transmitted to naïve MKPV-free mice housed in a cage that had previously housed mice shedding MKPV. Two MKPV contaminated cages, each housing 1 female NOD.Cg-Prkdc$^{scid}$Il2rg$^{tm1Wjl}$/SzJ (NSG) mouse inoculated 6 mo prior with MKPV as described below, were used. Soiled bedding from each contaminated cage was discarded and the cage was rinsed with warm tap water then inverted over paper towels inside a biologic safety cabinet for at least 2 h until completely dry. Autoclaved bedding, enrichment, food, and water were added, as described below, and 2 naïve Tac:SW mice were housed in each cage. Each week, the above cage rinsing process was repeated for newly provided soiled cages so that each pair of Tac:SW mice was exposed to the rinsed cage of an MKPV-infected NSG mouse weekly for a total of 6 wk. Fourteen wk after the initial exposure event, urine was collected from all 4 Tac:SW mice and tested for MKPV by PCR (Idexx Bioanalytics, Columbia, MO).

***Final study.*** Forty-two, 17- to 19-wk-old C57BL/6NCrl female mice that were shedding MKPV in their urine (confirmed by PCR) housed in 15 cages (3 or 4 mice per cage), changed weekly, were used to generate MKPV-contaminated cages ($n = 60$) over four weeks. Each MKPV-contaminated cage was randomly assigned to 1 of 3 groups: 1) bedding removed with no further cage processing (BR; $n = 20$); 2) bedding removed followed by sanitization in a tunnel washer (TW; $n = 20$); or, 3) cages processed as described in group 2 followed by autoclaving (WA; $n = 20$). Groups of 4 MKPV-naïve CD1 mice ($n = 30$ groups of 4 mice; 10 groups/treatment) were housed in test cages for 1 wk. Because only 15 contaminated source cages were available per week, groups of naïve CD1 mice were housed in test cages from their assigned group on alternating weeks, so that five groups of mice per treatment group were housed in test cages per week (Figure 1). Mice not housed in test cages were housed in stock autoclaved caging, prepared as described below.

After soiled bedding was removed from MKPV-contaminated cages, a cotton applicator (Pigeon Corporation, Tokyo, Japan) was used to swab the interior perimeter of the cage bottom and then from corner to corner in an "X" pattern. Swabs were stored at −80 °C before conducting MKPV PCR on an extract from each swab. After collection of the PCR sample, a test for organic material (UltraSnap Surface ATP Test, Hygiena, Camarillo, CA) was performed on a cage swab sample collected as described for PCR. Samples were processed within 1 h of collection using a commercially available luminometer (SystemSURE II, Hygiena, Camarillo, CA).

Cages assigned to the BR group were not processed further. Autoclaved bedding, enrichment, food, water, wire bar lid, and filter top, as described below, were added to each cage together with 4 MKPV-naïve Crl:CD1(ICR) mice. After bedding removal, cages assigned to the TW and WA groups were sanitized without chemicals in a tunnel washer (Basil 6300, Steris, Mentor, OH) operating with a belt speed of 6 linear ft/min. A data logger (OM-CP-HITemp140, Omega Engineering, Bridgeport, NJ) was run through the tunnel washer to confirm time and temperature. The washer provided a 40-s cold water prewash, a 40-s wash at 185 °F, first rinse at 180 °F for 30 s, and a 20-s final rinse of at least 190 °F, followed by drying with an air knife blow-off system at 2200 CFM (62.30 $m^3$/min.) air flow at 165 °F for 1 min. The final rinse temperature was confirmed using temperature-sensitive tape (Thermostrip, Cole-Parmer, Vernon Hills, IL) placed on the flat surface of a wire-bar lid immediately before processing cages through the tunnel washer, as well as the review of real-time operational parameters displayed on the washer's operator interface screen. After washing, cages in the TW group were tested for MKPV and organic material as described previously, and autoclaved bedding, enrichment, wire bar lid, food, and water, processed as described below, were added to each cage together with 4 MKPV-naïve CD1 mice. WA group cages were fitted with an autoclaved filtertop after retrieval from the tunnel washer. The filtertop was taped in place at both ends with heat-sensitive autoclave tape (Medline, Mundelein, IL), and the cage was autoclaved (Century SLH Scientific, Steris, Mentor, OH) as described below. After autoclaving, cages in the WA group were tested for MKPV and organic material as described above, and autoclaved bedding, enrichment, wire bar lid, food, and water, as described below, were added to each cage together with 4 MKPV-naïve CD1 mice.

The cage wash and housing processes described were repeated so that each group of CD1 mice was housed in a cage from the same treatment group 14 d later for a second 1-wk exposure period, resulting in testing a total of 60 contaminated

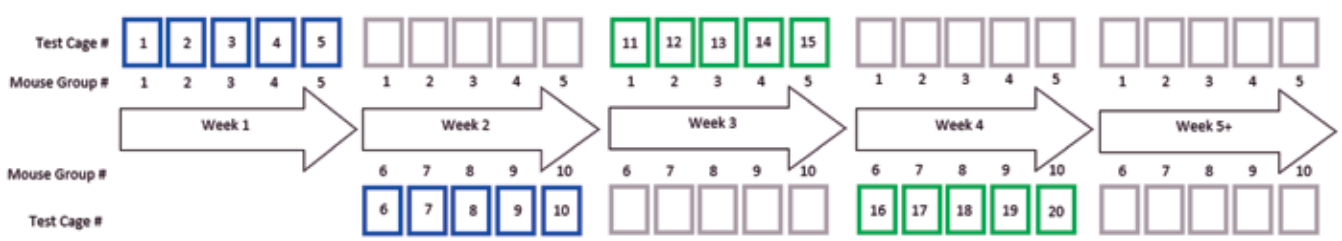


**Figure 1.** Schematic of study design. The image represents a single treatment group (WA, TW, or BR). Squares represent individual cages housing groups of 4 naïve CD-1 mice. Cages in blue represent the first set of 5 test cages to which each group of naïve mice was exposed. Cages in green represent the second set of test cages to which each group of naïve mice was exposed. Cages in grey are autoclaved and not contaminated with virus. A total of 10 groups of 4 naïve CD-1 mice, and 20 unique test cages, were utilized for each treatment group.

cages (20 cages/treatment group). For each treatment group, 5 groups of mice were housed in test cages on weeks 1 and 3, and the 5 remaining groups were exposed on weeks 2 and 4, for a total of 30 groups of mice (10 groups of mice/treatment group) (Figure 1). Mice were housed in autoclaved cages during the intervening weeks as well as after the second exposure period for the duration of the study.

Twelve ($n$ = 30), 17 ($n$ = 15), and 20 ($n$ = 15) wk after the first exposure event, 1 mouse per cage was euthanized by carbon dioxide asphyxiation and the left kidney was collected, stored at −80 °C, and tested for MKPV by PCR. Mice remaining in each cage after 20 wk were euthanized by carbon dioxide asphyxiation.

**Animals.** Five- to 6-wk-old female Swiss Webster (Tac:SW [SW]; Taconic Biosciences, Germantown, NY) mice ($n$ = 4) and 6- to 8-wk-old female Crl:CD1(ICR) (CD1, Charles River Laboratories, Senneville, Quebec) mice ($n$ = 120) were used in the pilot and final studies. C57BL/6NCrl female mice (B6; Charles River Laboratories, Senneville, Quebec), 17- to 19-wk-old ($n$ = 42) and NOD.Cg-Prkdc$^{scid}$Il2rg$^{tm1Wjl}$/SzJ female mice (NSG; Jackson Laboratories, Bar Harbor, ME), approximately 8 mo of age ($n$ = 2), were infected with MKPV as described below and used as the source of contamination to create MKPV-contaminated cages. All mice were free of mouse hepatitis virus, Sendai virus, mouse parvovirus, minute virus of mice, murine norovirus, murine astrovirus 2, pneumonia virus of mice, Theiler meningoencephalitis virus, epizootic diarrhea of infant mice (mouse rotavirus), ectromelia virus, reovirus type 3, lymphocytic choriomeningitis virus, K virus, mouse adenovirus 1 and 2, polyomavirus, murine cytomegalovirus, mouse thymic virus, Hantaan virus, mouse kidney parvovirus, *Mycoplasma pulmonis*, *Citrobacter rodentium*, *Salmonella* spp., *Filobacterium rodentium*, *Clostridium piliforme*, *Corynebacterium bovis*, fur mites (*Myobia musculi*, *Myocoptes musculinis*, and *Radfordia affinis*), pinworms (*Syphacia* spp. and *Aspicularis* spp.), and *Encephalitozoon cuniculi* when the studies were initiated, as determined by testing naïve outbred SW mice exposed repetitively to soiled bedding from cages housing mice in the colony.

**Husbandry and housing.** Mice were maintained in individually ventilated polysulfone cages with stainless-steel wire-bar lids and filter tops (number 19, Thoren Caging Systems, Hazelton, PA) on aspen chip bedding (PWI Industries, Quebec, Canada) at a density of no greater than 4 mice per cage. Each cage was provided with a bag constructed of Glatfelter paper containing 6 g of crinkled paper strips (EnviroPak, WF Fisher and Son, Branchburg, NJ) for enrichment. Mice were fed a natural ingredient, closed source, autoclavable diet (5KA1, LabDiet, Richmond, VA) and provided reverse osmosis acidified (pH 2.5 to 2.8 with hydrochloric acid) water in polyphenylsulfone bottles with stainless-steel caps and sipper tubes (Tecniplast, West Chester, PA) ad libitum. Cages that were autoclaved were subject to a pulsed vacuum cycle of 4 pulses at a maximum pressure of 12.0 psig, with sterilization temperature of 250.5 °F (121.39 °C) for 20 min, and a 10.0 inHg vacuum dry. Sterilization was confirmed by tape color change and review of the postcycle chamber conditions. Water bottles were autoclaved at a temperature of 250 °F (121 °C) for 45 min with a purge time of 10 min. Cages were changed every 7 d within a class II, type A2 biologic safety cabinet (BSC; LabGard S602-500, Nuaire, Plymouth, MN). The rooms were maintained on a 12:12-h light:dark cycle, relative humidity of 30% to 70%, and room temperature of 72 ± 2 °F (22 ± 1 °C). The animal care and use program at Memorial Sloan Kettering Cancer Center (MSK) is accredited by AAALAC, and all animals are maintained in accordance with the recommendations provided in the *Guide*.[6] All animal use in this investigation was approved by MSK's IACUC in agreement with AALAS' position statements on the Humane Care and Use of Laboratory Animals and Alleviating Pain and Distress in Laboratory Animals.

**MKPV-infected mice.** MKPV-infected NSG (pilot study; $n$ = 2) or B6 (main study; $n$ = 54) mice were used to contaminate cages. Mice had been experimentally inoculated approximately 6 (pilot study) or 2.5 (main study) months earlier with MKPV for independent studies evaluating the biology of MKPV. Briefly, viral stock (MKPV substrain MSK-WCM2015-3781-1-2) was created by thawing and homogenizing frozen kidney obtained from naturally infected NSG mice with histologically and PCR confirmed inclusion body nephritis (IBN) caused by MKPV infection. The homogenate was resuspended in 1× phosphate-buffered saline (PBS), passed through a sterile 0.22 µm filter, and then centrifuged at 626 x g for 5 min. Supernatant was collected, aliquoted, and stored at −80 °F. This viral stock was thawed once, diluted to 1:100 using sterile PBS, aliquoted into individual portions, and stored at −80 °F until used. At the time of inoculation, individual aliquots were thawed and administered to each mouse via oral gavage (200 µL viral stock) and intranasally (25 µL of viral stock per nostril; 50 µL total volume).

**PCR assay.** A proprietary real-time fluorogenic 5′ nuclease PCR assay specifically targeting *Rodent chaphamaparvovirus 1* was used to determine the presence of MKPV in swab extracts and kidneys. Samples that amplified during initial testing were retested using DNA isolated from a retained lysate sample to confirm the original finding. A positive result was reported when the retested sample was confirmed positive. To monitor for successful DNA recovery after extraction and to assess whether PCR inhibitors were present, a nucleic acid recovery control assay was also performed. Exogenous algae DNA was added to the sample lysis before extraction to yield approximately 200 copies of isolated nucleic acid per reaction well and a separate real-time PCR assay targeting the algae sequence was performed. Nucleic acid recovery control assays for samples that demonstrated greater than a log10 loss of template copies compared with control wells were diluted 1:4 and retested, reextracted, or both before accepting results as valid.

**Statistical analysis.** The MKPV incidence proportion of cage or renal PCR between treatment groups, weeks of exposure, and the number of infected mice per source cage were compared using Fischer exact test. Weekly variances in copy number detected in each of the 3 treatment groups were compared using a one-way ANOVA. The average number of days after inoculation of contaminated source mice between PCR positive and negative cages was examined using a 2-tailed $t$ test. Pretreatment RLU from each of the 3 treatment groups were compared using a 1-way ANOVA; when comparing the RLU values between 2 groups, a 2-tailed $t$ test was used. All analyses were performed using statistics software (Graph Pad Prism 9.1.0, La Jolla, CA). A $P$ value of less than or equal to 0.05 denoted statistical significance. Data are presented as mean ± standard deviation (SD).

## Results

**Pilot study.** Urine samples from all 4 (100%) SW mice exposed to MKPV contaminated caging were MKPV PCR positive when tested 14 wk after initial exposure.

**Cage swab PCR results.** Thirty-eight of 60 (63%) pretreatment cages were positive for MKPV by PCR (Table 1). No significant difference was found in the number of PCR positive cages when comparing cages containing 3 mice (14 of 24 cages; 58%) to those housing 4 mice (24 of 36; 67%) mice. Similarly, no significant

differences were found in mean copy number when comparing positive cages housing 3 mice (27 ± 25) to those housing 4 mice (52 ± 86). No significant difference was found in the incidence proportion of MKPV positive cages assigned to the BR, TW, and WA groups for either the first (Pre1; 70%, 40%, and 50%, respectively) or second (Pre2; 80%, 70%, and 70%, respectively) exposure event. The number of PCR-positive cages was significantly lower during the first week of sampling, when infected mice were on average 68 d after inoculation, as compared with the fourth week of sampling, when mice were on average 88 d after inoculation ($P$ = 0.005) (Table 1). The mean MKPV copy number in PCR positive cages was also significantly higher during the fourth, as compared with the first ($P$ = 0.006), second ($P$ = 0.027), and third ($P$ = 0.006) sampling weeks (Table 1). The mean number of days after inoculation of the source mice was significantly higher for cages that were positive for MKPV by PCR (81 ± 8.17) as compared with cages that were negative (74 ± 7; $P$ = 0.002). All TW and WA cages were MKPV PCR negative after treatment (Table 2).

**MKPV transmission.** Kidneys from all mice in the TW or WA groups were PCR negative for MKPV at 12, 17, and 20 wk after initial exposure. In contrast, renal tissue collected from mice in the BR group was MKPV PCR positive at 12 (3 of 10; 30%), 17 (1 of 5; 20%), and 20 (5 of 5; 100%) weeks after exposure (Table 2). Extracts from swabs collected from 5 cages in the BR group were MKPV PCR positive only during 1 of the 2 exposure events, while the remaining 5 cages were PCR positive during both. By 20-wk after exposure, the number of mice with MKPV PCR positive renal tissue (3 of 5; 60%) did not differ between mice exposed to either 1 or 2 PCR-positive soiled cages (Table 2). MKPV-positive cages from the BR group had a significantly greater likelihood (incidence proportion) of mouse infection with MKPV (70%) as compared with the likelihood of infection of mice housed in pretreatment MKPV-positive cages in the TW (0%; $P$ = 0.004) or WA (0%; $P$ = 0.004) groups.

**ATP swabs.** The mean pretreatment ATP luminescence obtained from the soiled cage swabs collected for the BR, TW, and WA cage groups did not differ significantly (26.5 ± 13.7, 30.5 ± 16.1, and 26.1 ± 17.3 RLU, respectively). The difference in ATP luminesce between dirty pre-treatment cages and clean post-treatment cages within the TW or WA group (TW = 0.45

**Table 1.** Pretreatment cage swab PCR results based on time postinoculation of source mice

| | Source mice days after inoculation | Positive | Negative | Percentage positive | Copy number of positive cages |
|---|---|---|---|---|---|
| Week 1 | 68 (65 – 72) | 6* | 9 | 24.83% | 9.4 ± 14.3* |
| Week 2 | 75 (72 – 79) | 10* | 5 | 66.67% | 21.4 ± 26.1* |
| Week 3 | 82 (79 – 86) | 8 | 7 | 53.33% | 9.52 ± 14.3* |
| Week 4 | 89 (86 – 93) | 14 | 1 | 93.33% | 67.93 ± 107 |
| Total | | 38 | 22 | 63.33% | |

Data for source mice days after inoculation are shown as mean (range). Data for copy number are shown as mean ± SD. * indicates statistically significant difference compared with week 4 ($P \leq 0.05$).

**Table 2.** MKPV PCR results for cage swabs and renal samples

| Bedding Removed (BR) Group | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Cage | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Pre1 | – | +(12) | +(12) | +(12) | +(25) | +(25) | – | +(25) | – | +(12) |
| Pre2 | +(25) | +(12) | – | +(50) | – | +(6) | +(12) | +(201) | +(25) | +(6) |
| 12 wk | – | – | +(811) | – | – | – | +(404) | +(215,443) | – | – |
| 17 wk | + (3,275) | – | – | – | – | NT | NT | NT | NT | NT |
| 20 wk | NT | NT | NT | NT | NT | + (13,219) | +(>1 × $10^7$) | +(811) | + (869,749) | + (3,275) |
| Tunnel Wash (TW) Group | | | | | | | | | | |
| Cage | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Pre1 | – | – | – | – | – | +(6) | +(25) | – | +(25) | +(100) |
| Post1 | – | – | – | – | – | – | – | – | – | – |
| Pre2 | – | +(25) | +(12) | – | +(12) | – | +(100) | +(25) | +(100) | +(12) |
| Post2 | – | – | – | – | – | – | – | – | – | – |
| 12 wk | – | – | – | – | – | – | – | – | – | – |
| 17 wk | – | – | – | – | – | NT | NT | NT | NT | NT |
| 20 wk | NT | NT | NT | NT | NT | – | – | – | – | – |
| Wash + Autoclave (WA) Group | | | | | | | | | | |
| Cage | 1 | 2 | 2 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Pre1 | +(25) | +(50) | – | – | – | – | +(25) | +(25) | – | +(50) |
| Post1 | – | – | – | – | – | – | – | – | – | – |
| Pre2 | +(6) | – | +(12) | – | – | +(404) | +(50) | +(25) | +(25) | +(25) |
| Post2 | – | – | – | – | – | – | – | – | – | – |
| 12 wk | – | – | – | – | – | – | – | – | – | – |
| 17 wk | – | – | – | – | – | NT | NT | NT | NT | NT |
| 20 wk | NT | NT | NT | NT | NT | – | – | – | – | – |

Estimated PCR copy number shown in parenthesis. Pre1 = first exposure pretreatment cage swab (first exposure occurred on week 1 for cages 1-5 in each group, and on week 2 for cages 6-10 in each group). Post1 = first exposure posttreatment cage swab. Pre2 = second exposure pretreatment cage swab (second exposure occurred on week 3 for cages 1-5 in each group, and on week 4 for cages 6-10 in each group). Post2 = second exposure posttreatment cage swab. 12 wk = renal sample collected 12 wk after the first exposure. 17 wk = renal sample collected 17 wk after the first exposure. 20 wk = renal sample collected 20 wk after the first exposure. NT = not tested

± 0.61 RLU, WA = 0.20 ± 0.41 RLU) was significant ($P < 0.0001$), but the difference in post-treatment ATP luminescence between groups was not.

## Discussion

This study indicates that exposing mice to MKPV-contaminated caging can result in infection of naïve mice and suggests that mechanical cage washing using equipment providing the exposure temperatures and durations evaluated in this study is sufficient to remove MKPV nucleic acid and prevent transmission of MKPV from contaminated caging to naïve mice. A risk of MKPV transmission via contaminated fomites was detected, as 7 of 10 cages of mice had at least one mouse test positive for MKPV at 17 or 20 wk after exposure to untreated contaminated cages for two 1-wk periods. The number of exposures to soiled cages that were positive for MKPV nucleic acid was not predictive of eventual MKPV infection in naïve mice, as the number of cages of mice that tested positive for MKPV was identical between those exposed to either 1 or 2 PCR-positive soiled cages. Swabs detected MKPV nucleic acid in most of the cages; however, all cages presumably should have been similarly contaminated with MKPV as they all contained MKPV-infected mice that were inoculated identically. Variation in the amount of urine absorbed by the bedding, variation in urine distribution within the cage, and minor variability in swabbing pattern may have contributed to variability in the amount of virus present on the swab.

Soiled cages were significantly more likely to test positive by cage swab PCR when the MKPV-inoculated source mice were at a later stage of infection. The inoculated mice used in this study were 12 to 13 wk after inoculation during the final exposure event, when the percentage of positive cages, as well as the average viral copy number of PCR positive cages, were significantly greater than during the initial exposure event, where infected source mice were 9 to 10 wk after inoculation. This difference suggests that MKPV is more likely to be transmitted by a soiled cage when infected mice are at a later stage of infection.

None of the mice from cages treated by cage wash alone or cage wash and autoclaving tested positive for MKPV. In contrast, most mice housed in cages without treatment tested positive by 20 wk after exposure. This supports the conclusion that MKPV transmission is effectively interrupted by the cage wash process when exposed to the washing conditions provided by the tunnel washer used in this study. In contrast, the murine *Protoparvovirus* MVM is stable at 70 °C for 1 h and remains infectious after exposure to 80 °C for the same period of time.[2] The *Protoparovirus* MPV remains infectious after being exposed to consecutive heat exposures to 176 °F (80 °C) for 5 to 10 s and 275 °F (135 °C) for 90 s.[1] However, despite demonstrated thermal resistance, cage wash at 110 °F (43.3 °C) or 180 °F (82 °C) with detergents prevented MPV transmission.[3,4] The thermal resistance of MKPV has not been evaluated directly, and therefore we cannot definitively conclude that the disruption in MKPV transmission in the present study is due to thermal inactivation of the virus, physical removal of the virus by washing, or both. The absence of any detectable viral nucleic acid on posttreatment cage swabs suggests that physical removal of the virus from the surface of the cage does occur.

As expected, both cage wash alone and cage wash with autoclaving significantly reduced the amount of organic material in the cages, as measured by ATP luminescence. No significant difference was detected in ATP reduction between cage wash alone and cage wash with autoclaving. To our knowledge, this is the first study directly comparing ATP luminescence outcomes between cages sanitized by a tunnel washer and cages sanitized by a tunnel washer and then sterilized by autoclaving.

A limitation of this study was the unequal distribution of MKPV PCR positive soiled cages among treatment groups due to the time required to obtain PCR results. Cages were randomly assigned to a treatment group immediately after initial cage swabbing, before obtaining PCR results. As we could not distribute cages equally between groups based on cage swab PCR positivity; we ensured that cages were randomly distributed between groups and that each group received equal numbers of soiled cages housing either 3 or 4 MKPV-infected mice. Despite randomization, groups received an unequal number of PCR positive cages, with group WA receiving 12, group TW receiving 11, and group BR receiving 15 positive cages. However, the difference in incidence proportion of MKPV positive caging assigned to each of the 3 treatment groups during the first (Pre1) or second (Pre2) exposures was not significantly different. Another study limitation was the termination of select cages of mice at 17 wk after initial exposure. Based on the pilot study, which resulted in 100% infection of mice within 14 wk of exposure to soiled caging, we initially selected an end point of 17 wk after the first exposure, and 15 wk after the second exposure. However, based on the relatively low number of cages that tested positive at the 12-wk time point, we decided to extend the terminal timepoint to 20 wk after exposure to increase the likelihood of detecting all infected mice. Not all mice could be tested at the 20 wk time point because this decision was made after the first cohort of mice had reached their terminal 17-wk time point.

Our institution does not use chemical detergents during the cage wash process. We can therefore conclude that the physical removal of material on the cage surface with pressurized water, as well as the exposure to high temperatures employed during the cage wash process, were adequate to prevent transmission of MKPV without the need to use chemical detergents or autoclave caging. By avoiding the use of chemicals or autoclaving, cages experience less thermoplastic degradation which may reduce the potential exposure of research animals to adverse chemicals such as BPA.[7]

To summarize, we can conclude that mechanical washing alone should be sufficient to adequately sanitize MKPV positive caging. Our final rinse temperature (190 °F) is higher than the traditional final rinse temperature of 180 °F suggested by the *Guide*.[6] This elevated set point ensures that any temperature deviations during use of the tunnel washer throughout the workday would present a reduced risk of the cage wash temperature dropping below the recommended 180 °F. Although investigating the role of the wash/rinse water temperatures in eliminating MKPV on cages was not an aim of this study, evaluation of this variable would expand the applicability of these findings to more animal resource programs.

## References


1. **Adams SC, Myles MH, Tracey LN, Livingston RS, Schultz CL, Reuter JD, Leblanc M.** 2019. Effects of pelleting, irradiation, and autoclaving of rodent feed on MPV and MNV infectivity. J Am Assoc Lab Anim Sci **58:**542–550. https://doi.org/10.30802/AALAS-JAALAS-18-000142.
2. **Boschetti N, Wyss K, Mischler A, Hostettler T, Kempf C.** 2003. Stability of minute virus of mice against temperature and sodium hydroxide. Biologicals **31:**181–185. https://doi.org/10.1016/S1045-1056(03)00037-X.
3. **Compton SR, Macy JD.** 2015. Effect of cage-wash temperature on the removal of infectious agents from caging and the detection of infectious agents on the filters of animal bedding-disposal cabinets by PCR analysis. J Am Assoc Lab Anim Sci **54:**745–755. Available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4671790/.

4. **Compton SR, Paturzo FX, Smith PC, Macy JD.** 2012. Transmission of mouse parvovirus by fomites. J Am Assoc Lab Anim Sci **51:**775–780. Available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3508181.
5. **Edmondson EF, Hsieh W-T, Kramer JA, Breed MW, Roelke-Parker ME, Stephens-Devalle J, Pate NM, Bassel LL, Hollingshead MG, Karim BO, Butcher DO, Warner AC, Nagashima K, Gulani J.** 2020. Naturally acquired mouse kidney parvovirus infection produces a persistent interstitial nephritis in immunocompetent laboratory mice. Vet Pathol **57:**915–925. https://doi.org/10.1177/0300985820953500.
6. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
7. **Krishnan AV, Stathis P, Permuth SF, Tokes L, Feldman D.** 1993. Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving. Endocrinology **132:**2279–2286. https://doi.org/10.1210/endo.132.6.8504731.
8. **Lee Q, Padula MP, Pinello N, Williams SH, O'Rourke MB, Fumagalli MJ, Orkin JD, Song R, Shaban B, Brenner O, Pimanda JE, Weninger W.** 2020. Murine and related chapparvoviruses are nephro-tropic and produce novel accessory proteins in infected kidneys. PLoS Pathog **16:**e1008262. https://doi.org/10.1371/journal.ppat.1008262.
9. **McGaving D.** 1987. Inactivation of canine parvovirus by disinfectants and heat. J Small Anim Pract **28:**523–535. https://doi.org/10.1111/j.1748-5827.1987.tb01446.x.
10. **Pattison JR, Patou G.** Parvoviruses. In: Baron S, editor. Medical microbiology. Galveston (TX): University of Texas Medical Branch at Galveston.
11. **Pénzes JJ, Söderlund-Venermo M, Canuti M, Eis-Hübinger AM, Hughes J, Cotmore SF, Harrach B.** 2020. Reorganizing the family Parvoviridae: a revised taxonomy independent of the canonical approach based on host association. Arch Virol **165:**2133–2146. https://doi.org/10.1007/s00705-020-04632-4.
12. **Roediger B, Lee Q, Tikoo S, Cobbin JCA, Henderson JM, Jormakka M, O'Rourke MB, Padula MP, Pinello N, Henry M, Wynne M, Santagostino SF, Brayton CF, Rasmussen L, Lisowski L, Tay SS, Harris DC, Bertram JF, Dowling JP, Bertolino P, Lai JH, Wu W, Bachovchin WW, Wong JJL, Gorrell MD, Shaban B, Holmes EC, Jolly CJ, Monette S, Weninger W.** 2018. An atypical parvovirus drives chronic tubulointerstitial nephropathy and kidney fibrosis. Cell **175:**530–543.e52. https://doi.org/10.1016/j.cell.2018.08.013.
13. **Saknimit M, Inatsuki I, Sugiyama Y, Yagami K.** 1988. Virucidal efficacy of physio-chemical treatments against coronaviruses and parvoviruses of laboratory animals. Jikken Dobutsu **37:**341-345. https://doi.org/10.1538/expanim1978.37.3_341.
14. **Scott FW.** 1980. Virucidal disinfectants and feline viruses. Am J Vet Res **41:**410–414.
15. **Watson J.** 2013. Unsterilized feed as the apparent cause of a mouse parvovirus outbreak. J Am Assoc Lab Anim Sci **52:**83–88. Available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3548206/.