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The use of PCR and environmental swabs to identify mouse parvovirus post decontamination

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Abstract

Due to a history of recurrences of Mouse Parvovirus (MPV) outbreaks, a novel procedural solution to environmentally screen previously contaminated rooms was explored. The authors wanted to evaluate a rapid, reliable means to test the environment without using animals by investigating the use of environmental swabs to detect residual MPV after rooms were depopulated and decontaminated. Polymerase Chain Reaction (PCR) was performed on environmental swabs to determine the presence of DNA that could be indicative of residual MPV in the affected rooms. The absence of MPV DNA demonstrated that the rooms were adequately decontaminated, and the likelihood of a re-infection was minimal after repopulation. The use of PCR to confirm the absence of environmental MPV has been an excellent method to determine the effectiveness of post outbreak decontamination.

Key words: Mouse Parvovirus (MPV), Polymerase Chain Reaction (PCR), Environment, Decontamination, Replacement

Introduction

MPV is a non-enveloped single-stranded DNA virus (18–25 nm) and is moderately contagious. Mice are infected primarily by direct contact with virus shed in feces or urine. Infections are usually chronic with extended duration of virus shedding. (Jacoby *et al*., 1996) This virus is a contributing factor to complications in studies involving immunology and tumor growth in our facility. (Jacoby *et al*. 1995; McKisic *et al*. 1996; Besselson *et al*. 2006) For example, MPV infections could result in the interference of experimental compounds such as CTLA4, working at the T cell level in immunology studies (McKisic *et al*. 1996; Macy *et al.*, 2009) Due to the nature of our research (oncology/immunology) and the impact of closing rooms for several weeks while using sentinels to screen for the presence of MPV, we had to evaluate a more reliable, timely process to repopulate previously MPV contaminated rooms.

It is critical that animal health surveillance programs are able to detect adventitial viruses, and in our case, we wanted to detect MPV in the animals and environment. Standard sentinel testing in our facility includes a combination of serology screening, PCR of the mesenteric lymph nodes (MLN) and/or spleen, bacteriology, parasitology and necropsy. However, environmental monitoring practices in conjunction with routine procedures are emerging. Compton, *et. al.*, researched the efficacy of detecting various microbial agents on gauze filters placed on exhaust pre-filters of individually ventilated cage (IVC) animal racks. Mouse hepatitis virus, Sendai Virus, MPV and Helicobacter sp. were detected by RT-PCR and PCR analysis of the gauze filters; thereby, indicating that viruses can be detected on materials other than animal tissues (Compton *et al*. 2004).

One of the speculations for MPV recurrences in our facility involved the effectiveness of the decontamination process, particularly sanitization and disinfection of room air vents and ventilation systems, hoods, floors, surfaces and equipment (Charles River Laboratories, 2009).

Parvoviruses can remain infectious in the environment for long periods and may persist on environmental surfaces and fomites such as counter tops, equipment, supplies, and corners and crevices of rooms, and especially cage components such as soiled bedding, food, wire bars, filter tops, nesting material and shelters (Clifford and Watson, 2008; Macy *et al*. 2009; Compton *et al*. 2012) In order to investigate the effectiveness of our decontamination procedures, we performed and evaluated environmental swab collection and PCR testing of the affected rooms, targeting surfaces that were difficult to clean or sanitize, and areas of high traffic flow (Macy *et al*. 2009).

Materials and Methods

Sample Collection

Rayon-tipped swabs, BactiSwab® Dry (Remel, Lenexa, KS) were used for sample collection. Environmental swabs were stored at room temperature in a cool, dry environment until they were ready for use. The tip of the swab was dipped into sterile water and then swabbed over a targeted location. One swab was used to cover an area of up to 20 cm^2 and was wiped over the location in a back and forth motion for 10 seconds. The swab was then recapped, given a unique identification number and placed in a plastic bag. Each identification number was recorded on a log sheet with each location.

Animal Housing

Mice in conventional rooms were housed as five animals per cage in non-autoclaved polycarbonate open top hanging cages (Lab Products, Seaford, DE), on non-autoclaved racks (Lab Products, Seaford, DE). The racks held 98 boxes and Edstrom automatic watering (chlorinated, deionized water) was provided *ad libitum*. Non-autoclaved bedding (Shepherd Specialty Paper/W.F. Fisher and Son, Somerville, NJ) was used, and nestlets (Ancare, Bellmore, NY) were placed in cages for enrichment. Mice were fed non-irradiated Teklad Global 2018 Rodent Diet (Harlan, Frederick, Maryland) and were housed under normal macro environmental conditions (Temp 72 +/- 2°F: RH 50% +/-10%, light:dark cycle 12:12, >15 air exchanges per hour of 100% fresh filtered air) in compliance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011) All animals were received and used under an approved IACUC protocol. The facility is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

Confirmation of MPV

MPV infection was characterized by ICR sentinel mice testing positive by serological evaluation, followed by confirmation with additional positive serology and MLN PCR samples taken from stock and study mice in the same room. The most commonly used strains for stock and study animals included DBA/2 and SJL mice. After a room was confirmed to have MPV, environmental swab samples were collected from various locations. Some swabs were collected from the animal cages by wiping the entire inside bottom of the cage, with dirty bedding present, in a back and forth motion for 10 seconds. Animal racks were swabbed near the location of the sipper where bedding material would routinely accumulate. Animal room exhaust air vents were swabbed over five randomly selected slats, reaching far enough through the slat to touch the filter material. Randomly selected locations of floors, doors, walls and equipment were also swabbed to confirm that MPV could be detected by *in vitro* methods. These samples were sent to an outside laboratory (Charles River Laboratories, Wilmington, MA) for PCR analysis.

Sanitization and Decontamination Process

Any room that was identified to have MPV positive animals determined by serology and MLN PCR was broken down, sanitized, and decontaminated. The first step in the break down and decontamination process included the euthanasia of any remaining stock and sentinel animals, as well as animals not critical to the completion of studies. Animals that were critical for study were transferred to a room in a separate area of the facility specifically designated for quarantine and were maintained under conventional housing practices as previously described. Staff was required to don jumpsuits and other personal protective equipment (PPE) to enter the quarantine room and dispose of all PPE in special waste containers maintained within the room upon exiting. Staff was not permitted to enter any other rodent area after working in the quarantine area.

Equipment/Caging

All movable equipment, caging and animal racks within the MPV positive room underwent a decontamination process. This included sanitizing the equipment within the contaminated room by fogging with one round of Virkon® S (DuPont, Alpharetta, GA). The equipment, cages, and racks were then covered and taken to cage wash for additional sanitization. Since these rooms involved standard conventional housing practices, the equipment, caging and racks were not autoclaved prior to being placed back into use. Sani-plex® 128 (Quip Laboratories, Wilmington, DE) was used for routine sanitization of all walls, floors, hoods and surfaces, in addition to wiping down equipment with Virkon® S.

Rooms/Laboratories

The animal rooms and laboratories also underwent a decontamination process which included three alternating rounds of fogging chemicals, using Virkon® S and Clidox® S, (Pharmacal, Naugatuck, CT). Any stationary equipment remaining in the room was disassembled and laid out in a position to receive contact during fogging. This equipment was rotated so that all surfaces came into contact with the disinfectant. When the decontamination process for the room and the equipment was completed, environmental PCR swab samples were collected again to determine if DNA viral material had been eliminated from the environment.

Results

In 2006, we began assessing if environmental swabs could detect the presence of MPV. We initially evaluated two rooms that contained MPV positive animals. Swabs were collected from areas such as the floor, doors, walls, animal cages,

animal racks, animal room exhaust air vents and equipment. Approximately 66% (4/6) and 83% (5/6) of environmental swabs tested positive for MPV by PCR prior to room decontamination during the trial period in these two rooms.
After evaluating and confirming that the procedures for collecting environmental swabs could detect MPV DNA, this became our standard method for screening the decontamination process after an outbreak. As previously described, the process included quarantine of animals critical to the completion of an existing study, depopulation of animals that were no longer required, as well as sanitization and decontamination of an infected room and equipment. After the process was completed, testing was performed with environmental swabs using PCR to identify MPV DNA.

In our experience, most swabs were negative after the initial decontamination process. However, in some cases, 10-35% of swab samples tested positive for MPV even after the initial decontamination process (Table 1, Rooms 1, 3 and 7). In these rooms, swabs were collected from locations such as floors, doors, walls, animal racks, exhaust air vents and equipment such as cleaning implements, rolling storage units, computer carts, anesthesia chambers and supplies. As demonstrated in these rooms, environmental swabs and PCR testing indicated that MPV DNA had not been completely removed from the environment after the initial decontamination process. These rooms underwent repeated rounds of the sanitization and decontamination procedures until all swab results were negative. This has helped to considerably reduce the number of animals (sentinel, stock, study) used for screening MPV in our facility by over 65%, from over 4,500 animals in 2005 to approximately 1,600 animals in 2012

Table 1. A history of environmental swab sample results

Room	Date	Environmental swab sample type	Total number	Number positive
Room 1	3/9/07	Post decontamination	6	2 (33.3%)
	4/2/07	Post decontamination round 2	6	0
Room 2	2/5/07	Post decontamination	6	0
Room 3	2/6/08	Post decontamination	13	2 (15.4%)
	3/17/08	Post decontamination round 2	18	2 (11.1%)
	4/25/08	Post decontamination round 3	12	0
Room 4	8/15/08	Post decontamination	15	0
Room 5	3/25/09	Post decontamination	6	0
Room 6	9/27/10	Post decontamination	8	0
Room 7	2/28/11	Post decontamination	10	2 (20%)
	3/7/11	Post decontamination round 2	10	0

Based on our experience, various locations within rooms have been identified as areas commonly contaminated with MPV DNA both pre and post decontamination of the room. These locations include animal room exhaust air vents (67%), walls (67%), flow hoods/change stations (60%), animal racks and caging (50%), floors and/or floor drains (44%), and various equipment including cleaning implements such as brooms and squeegees, rolling carts, and a computer foot pedal maintained in the animal and anterooms (36%). We identified these locations as the most common areas where MPV DNA was detected and we targeted these sites when collecting environmental swabs from an MPV positive room. Other areas that were screened and did not test positive included doors, waste receptacles, supply drawers, and supplies.

Additionally, we encountered an outbreak of RMV, Rat Minute Virus (also a parvovirus) and used environmental screening and PCR testing to identify this virus within various locations in these rooms. The outbreak was first identified in one of three sentinel rats in a conventional rat room (the same housing procedures as described in mice were used in the rat room). Additional testing one week later revealed 3/6 positive serology samples and 6/6 positive MLN PCR samples from sentinel rats. An additional 2/6 positive serology samples and 6/6 positive MLN PCR samples were indentified in non-sentinel animals. Environmental swabs were collected from the RMV positive room and two adjoining rooms. Positive samples were obtained in 8/28 environmental swab samples. The rooms underwent the decontamination process. This was followed by another round of environmental swab sampling which revealed 2/22 positives. This indicated that the first round of decontamination was inadequate. The rooms underwent a second round of decontamination and environmental swabbing resulting in 0/18 positive samples. The targeted locations for RMV were the same as the locations for MPV.

Discussion

In the past, sentinel animals were used as the environmental monitor of the post outbreak decontamination status of a room previously positive for MPV. After the room had been depopulated, sanitized and decontaminated, sentinel animals were placed into the room. At the end of the six week period, sentinel animals were euthanized and a necropsy was performed. However, this was time consuming and unreliable, leading us to seek a better alternative. We decided to evaluate the reliability of using environmental swabs as a method for detecting residual MPV DNA within a given area that had tested positive. (Macy *et al.* 2009). A series of environmental swabs collected from MPV positive rooms proved to be successful in identifying the virus. We also had success in decontaminating an area, screening with environmental swabs, and confirming that the decontamination had been successful in removing MPV DNA from the environment. It is important to note that the PCR test for MPV does not discern between live or dead virus. The test only confirms the presence of MPV DNA in the environment(Besselson *et al*. 2000; Besselson *et al*. 2006) We have treated all positive results as live viral particles and attributed the findings to insufficient sanitization methods. In these cases, the rooms underwent a repeated sanitization/decontamination process, until 100% of the environmental swab results were negative.
Because of the use of conventional housing in these areas, the authors realize that non-autoclaved caging and bedding and non-irradiated feed could also be a source of re-infection of Mouse Parvovirus (Clifford and Watson, 2008; Tradeline Inc. 2008; Reuter *et al*. 2011) This is not thought to be the case in our experience, as outbreaks would have been seen

more commonly in other conventional housing areas and in barriers where non-irradiated feed and bedding were used. In another company site, MPV outbreaks have also occurred in barriers where autoclaved caging systems and irradiated diet were used. It is also possible that the investigators working in this area were bringing in MPV contaminated equipment, biological samples or compounds. The investigators are required to screen biological materials to be used in animals through a PCR panel to detect the presence of any adventitial infectious agents.

The implementation of PCR testing of environmental swabs has proved to be beneficial to our animal health program. This procedure has assisted us in quickly bringing an MPV positive room back into service. This PCR technology has eliminated the use of sentinel animals for post decontamination monitoring for MPV. This has decreased "down time" (a time when rooms are vacant and not available for study use) from a period of nine or more weeks, to approximately four weeks. This procedure has increased our confidence in detecting MPV prior to repopulating a room.

Additionally, successfully detecting RMV demonstrates that there are potential applications for using environmental swabbing/PCR technology to screen for other viruses. This also demonstrates a refinement in diagnostic procedures by replacing sentinel animals with an *in vitro* method; thereby, saving animal lives.

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