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Detection and Control of Mouse Parvovirus

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Mouse parvovirus (MPV) remains a prevalent infection of laboratory mice. We developed 2 strategies to detect and control an active MPV infection over a 9.5-mo period. The first strategy used a test-and-cull approach in 12 rooms. After all cages corresponding to MPV-seropositive bedding sentinels were removed from the room, a naïve sentinel mouse was dedicated to every 2 to 3 rows per rack and received soiled bedding from these rows every 2 wk. All 12 rooms completed 3 consecutive negative rounds of targeted testing, which required an average of 20 wk. The second strategy used a modified quarantine approach to test unique mice that were critical for breeding. The process required removing selected cages from the seropositive rack and consolidating them to a single rack within the same room. All mice in these cages were tested by using MPV serology and fecal PCR. Cages were not moved, opened, or manipulated between sample collection and the availability of test results. The cages were relocated as a group to another room, because all mice were MPV negative. The mice were retested 3 wk after the initial testing, and all were MPV seronegative. Since the rooms were cleared 4 to 5 y ago, 7915 routine bedding sentinels and colony mice were tested from these rooms, all with negative results. These consistently negative MPV test results suggest that MPV was eliminated from these rooms, rather than driven down below the threshold of detection. These 2 strategies should be considered when confronting MPV infection.

Abbreviation: MPV, mouse parvovirus.

Mouse parvovirus (MPV), a lymphocytotropic parvovirus, is a prevalent viral infection of laboratory mice.[8,13] MPV was first isolated from cloned T cells, and pathogenesis studies confirmed its propensity to infect lymphoid tissues, including Peyer patches, thymus, spleen, peripheral lymph nodes, and mesenteric lymph nodes, with the latter 3 being sites of persistent infection.[6,9] MPV thus disrupts research through its effects on the host immune response, including aberrant T-cell proliferation responses and function and acceleration of T-cell–mediated rejection of tumors, skin allografts, and syngeneic skin grafts.[10,11]

MPV is a difficult infection to address because it is asymptomatic, persists for as long as 9 wk in tissues of immunocompetent mice,[6] and is believed to occur with low prevalence within a mouse colony. Fecal shedding of MPV is probably the major mechanism of virus transmission. Viral DNA is consistently detected by PCR analysis of feces for at least 2 wk after infection, although genotype influences the duration of shedding and the 'window' in which transmission occurs.[1,2,4,5,7,16] We and others have shown that peak levels of MPV DNA in the small intestine occur on postinfection day 5 to 10, and levels diminish substantially by week 2,[5,6] indicating a window of 2 wk or less for detection of intestinal MPV infection in many cases.

A threshold level of infectious MPV appears to be required for transmission to occur. Using experimental infection, we demonstrated that the threshold necessary for consistent transmission to sentinels exposed to an entire cage of soiled bedding was achieved at postinfection day 7 and 14 in Swiss Webster mice and BALB/c mice.[7] In addition, we and others have demonstrated that transmission of MPV to sentinels from immunocompetent mice through soiled bedding occurs only during the time of peak shedding (during first 2 wk of infection),[1,2,7,16] which is a fairly short window for transmission. Therefore, detection of MPV by fecal PCR does not always indicate that the mice are an immediate risk for transmitting MPV because infection is not always transmitted to other mice through soiled bedding or direct contact. The inconsistency with which transmission occurs can be demonstrated in mice exposed to the same amount of soiled bedding taken from the same source, even when the mice were housed and exposed while in the same cage. For example, in one study, exposure of sentinel mice to soiled bedding from cages housing MPV-infected mice at postinfection day 3, 7, or 14 resulted in seroconversion of just 60% of sentinel mice,[16] with only 1 of the 2 sentinels exposed to soiled bedding becoming seropositive in 35% of cages in which transmission occurred. Not surprisingly, the amount of MPV-contaminated soiled bedding influences seroconversion in sentinel mice. For example, a decrease in sensitivity of 29% at postinoculation day 7 and 58% at postinoculation day 14 was observed in sentinels exposed to 25 mL of MPV-soiled bedding compared with 400 mL of MPV-soiled bedding.[16] Other studies also have observed this decrease in sensitivity.[2,4]

In addition, the threshold level of MPV required to induce infection is influenced by the age of the mouse, its genotype, and the type of caging. Swiss Webster mice show a decline between 8 and 12 wk of age in susceptibility to MPV infection, with 12-wk-old mice requiring 20-fold more MPV to induce infection.[3] Furthermore, genotype is associated with a striking effect on susceptibility to MPV, affecting the threshold of virus required to induce infection. BALB/c mice are more susceptible to and sustain a more robust MPV infection than do C57BL/6 mice.[5] Other studies also demonstrate genotype-based differences in susceptibility, with the ID_{50} of MPV in BALB/c mice shown to be 1 to 2 logs lower than that in C57BL/6 mice.[3,12] In contrast, outbred mice (Swiss Webster, ICR) shed high levels of virus for the longest period of time and seroconvert more readily than do either BALB/c or C57BL/6 mice.[5] In general, C57BL/6 mice shed the lowest amount of MPV for the least amount of time, require higher virus doses to seroconvert, and have the longest lag time between exposure and seroconversion (more

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than 2 wk in many cases).[5] Little is known about the effect of immunodeficiency on MPV infection, except that SCID mice infected at 1 d of age shed high levels of MPV in their feces for 24 wk.[1] In addition, coinfection of BALB/c mice with MPV and mouse norovirus increases the level of MPV in feces, the small intestine, and mesenteric lymph nodes.[5]

These aspects of MPV infection raise important concerns about the sensitivity with which soiled bedding sentinel programs detect MPV infection within a colony and have been the basis for depopulating entire rooms to eliminate infection. Despite these potential concerns about MPV detection, we describe a successful test-and-cull strategy for MPV that uses 'targeted sentinels' and relies heavily on detection of small pockets of infection and a 'modified quarantine' strategy. Importantly, these strategies can be used as alternatives to depopulation of most or all mice within animal rooms in which MPV infection has been detected. This overall strategy was developed and tested as a result of a large-scale outbreak in a new animal facility. Many of the affected mice were deficient in some aspect of T cell and/or B cell immunity, although few were profoundly immunodeficient. In addition to implementation of the 2 strategies described, containment practices such as moving positive rooms lower in the room entry order, restricting movement of mice out of MPV-positive rooms, and the use of additional personal protective equipment to limit the spread of MPV were followed during the eradication process. Although prohibiting breeding and introduction of new mice into the positive rooms throughout the eradication process likely would have expedited our efforts, these practices were not feasible in the face of ongoing research needs, and the decision was made not to restrict breeding or the introduction of new mice into the facility. Despite these limitations, we successfully eliminated MPV from all positive rooms and have maintained the mice in these rooms free of MPV infection for over 4 y.

Materials and Methods

Mice. Female Swiss Webster mice (Tac:[SW]; age, 4 to 6 wk) to be used as sentinels were obtained from Taconic Farms (Germantown, NY). Vendor reports indicated mice were seronegative for ectromelia virus, murine rotavirus, lymphocytic choriomeningitis virus, mouse hepatitis virus, MPV, minute virus of mice, murine norovirus, pneumonia virus of mice, reovirus, Sendai virus, and *Mycoplasma pulmonis* and were free of bacterial and parasitic infections at the time of shipment. Mice housed in the rooms that contained MPV-positive mice were of mixed genetic background; approximately 70% were genetically engineered, with T or B cell disruption (or both) being the most common defect and C57BL/6 being the most common background strain of mouse. Most of the mice housed in the rooms that contained MPV-positive mice were generated inhouse, but many were introduced directly from multiple commercial vendors, imported from other institutions, and introduced after quarantine and testing or were relocated from other mouse rooms within the institution.

Facility, husbandry, and preventive medicine. An MPV outbreak occurred in 16 'basic service' rooms that contained 75 ventilated racks (140 cages per rack; 2 to 8 racks per room) in a new 52,000 ft^2 facility with 31 animal rooms, 21 procedure rooms, and a dedicated, central clean supply corridor and peripheral return corridor. The return corridor provided access for laboratory staff to the animals and procedure rooms as well the trafficking of soiled equipment to the wash center. The animal rooms had a positive pressure differential relative to the corridor, a 12:12-h light:dark cycle, 10 to 15 air changes hourly, room temperature of 22.2 ± 1.1 °C, and room humidity of 50% ± 10%. Mice were housed at an average density of 2.8 mice per cage in individually ventilated cages (model no. MD75JU140MVPSH, ACE, Allentown, NJ) containing corncob bedding (7092 or 7079, Harlan Teklad, Indianapolis, IN). Mice were fed rodent chow (Global 2018 or 2018S, Harlan Teklad) and drank hyperchlorinated (10 to 12 ppm) water ad libitum delivered by an automatic watering system with a shielded valve (A160, Edsrom, Waterford, WI).

Housing and husbandry for mice at our institution are divided into 3 levels of care, each with progressively increased microbiologic security, control, and surveillance. The first level—basic service—uses nonsterile cages, food (Harlan Teklad 2018) and bedding, and hyperchlorinated water is delivered through an automatic watering system. Cages (including all cage components, bedding, and food) are changed at 14-d intervals within a class II biological safety cabinet (SterilGARD, The Baker Company, Stanford, ME), and the forceps used to transfer mice are decontaminated between cages using chlorine dioxide solution (Clidox-S, Naugatuck, CT) at a 1:5:1 dilution. The second level of husbandry is called 'basic plus service' and uses autoclaved cages, food (Harlan Teklad 2018S) and bedding, with hyperchlorinated water delivered through an automatic watering system. This level of service was implemented to increase microbiologic security after the described MPV outbreak. The final level of care—'full service'—uses autoclaved cages, food (Harlan Teklad 2018S), and bedding, with hyperchlorinated water delivered in autoclaved water bottles. Both basic-plus- and full-service cages (including all cage components, bedding, and food) were changed at 14-d intervals within a class II biological safety cabinet, and the forceps used to transfer mice were decontaminated between cages by using chlorine dioxide solution at a 1:5:1 dilution. All MPV-positive rooms described in this report were basic-service rooms.

Routine bedding sentinels were used to detect pathogens. One cage containing 2 sentinel Swiss Webster mice (age, 4 to 6 wk) was placed on the bottom row on each side of the 140 cage ventilated racks. These cages received a systematic sampling (row by row) of soiled bedding from other cages on the rack to maximize contact with potential infection. The routine sentinel exposure protocol included the removal of approximately 25 mL (approximately 3 oz) soiled bedding from 2 rows (14 cages) on one side of a ventilated cage rack sequentially at the time of cage change. Because cages were changed at 14-d intervals, 10 wk were required for each side of a rack (10 rows) to be sampled. Rodent colonies were tested for adventitious infections quarterly. Bedding sentinels were replaced every 6 mo. Two screenings per year included a full panel of serology tests and testing for endo- and ectoparasites. Agents tested for by serology included mouse hepatitis virus, MPV, mouse rotavirus, Sendai virus, pneumonia virus of mice lymphocytic choriomeningitis virus, ectromelia virus, murine encephalomyelitis virus, mouse adenovirus, minute virus of mice, and reovirus. An abbreviated panel of serology tests for MPV, mouse hepatitis virus, and murine rotavirus was used for the alternate 2 screenings. Bacteriology (culture of gastrointestinal tract and nasopharynx) was performed on sentinels from full-service rooms to screen for *Bordetella bronchiseptica*, *Corynebacterium kutscheri*, *Klebsiella pneumonia*, *Klebsiella oxytoca*, *Mycoplasma pulmonis*, *Pasteurella pneumotropica*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Streptococcus* spp. (β-hemolytic), and *Streptococcus pneumoniae*. All animal care and experimental procedures were approved by the Yale Animal Care and Use Committee and were in accordance

with all federal policies and guidelines governing the use of vertebrate animals.

MPV infection. MPV-positive rooms were identified during sentinel screening over a 9.5-mo period between December 2004 and September 2005. The spread of MPV from rooms housing MPV-infected mice to 'clean' rooms was exacerbated because mice had been approved for relocation among a subset of rooms, without testing of individual mice being relocated, because the rooms were viewed to have similar risks and be of similar microbiologic status. In some cases, relocations occurred prior to detection of infection within the room. The MPV-positive rooms were identified either during routine quarterly sentinel screening or as a result of risk-based early testing of sentinels from rooms that had received mice from known MPV-positive rooms.

Strategies to address infection. Two real-time, complementary strategies (targeted sentinel testing and modified quarantine) to detect, control, and eliminate MPV were used.

The first strategy, targeted sentinel testing, used a test-and-cull approach. Once all cages corresponding to the initial seropositive bedding sentinels were removed from the room, remaining cages were spread out among the rows to ensure there was enough space to wean animals onto the same 2 to 3 rows as the parental cage; therefore weanlings could remain under surveillance by the same dedicated sentinel as their parents. A single naïve sentinel mouse (targeted sentinel) was dedicated to every 2 to 3 rows (14 to 21 cages) of each rack. The targeted sentinel received soiled bedding from these rows every 2 wk. Three weeks after the initial bedding exposure and approximately every 2 wk thereafter, the sentinels were tested for seroconversion to MPV. If any targeted sentinels became MPV seropositive, all cages corresponding to the seropositive sentinels were removed from the room and the sentinels were replaced. Therefore, it was important that colony mice not be moved from their designated rows to accurately narrow MPV-positive cages to specific areas of the rack. The 2-wk testing interval was delayed sometimes because of the difficulties in predicting the number of new targeted sentinels needed, because this quantity was contingent on the number of seropositive results of the previous 2-wk period. Targeted sentinels that did not seroconvert to MPV were euthanized when they were 5 to 6 mo old and were replaced with naïve 4-wk-old mice. Three consecutive MPV seronegative samples were required to elevate the room status from 'actively infected' to 'cleared with a history of MPV.'

On 2 separate occasions, a second strategy (modified quarantine) was used to expedite 'clearing' of small groups of unique breeding mice critical to propagating the desired genotypes. This strategy involved a modified quarantine approach, in which a subset of cages was identified, consolidated to a single rack within either the same room or a new room, and tested as described later.

The population of mice being tested by using modified quarantine was housed in a single room. However, this population of mice resulted from the combining of 2 rooms. The first of the 2 rooms had initial seropositive room sentinel results that were followed by 2 consecutive rounds of targeted sentinels with seropositive results (6 of 58 and 4 of 58 positive). The second room had seropositive room sentinel findings (4 of 44 positive), which were coincident with the second seropositive targeted sentinel finding in the first room. After the positive rows and racks were removed from each room, cages in these 2 rooms were combined into the first room, and the modified quarantine procedure was instituted. During the initial modified quarantine procedure, 75 cages of unique breeding mice were selected and placed on a separate rack within the same animal room. Each mouse in every cage was bled for MPV serology, and feces were collected and pooled from all mice within a cage for DNA extraction and MPV PCR. On completion of initial testing (usually within 3 d), cages were not moved, opened, or manipulated. Because all mice in all cages were negative for MPV by serology and PCR, the cages were relocated as a group into the new room. Three weeks later, the mice were rebled, MPV serology was performed, and these mice were used to repopulate the room.

Six months later, the same process was repeated with 161 breeding mice (58 cages) housed in the same animal room as those tested in the original modified quarantine; these mice were used as breeding stock to populate a new room.

Postinfection husbandry practices. During and after elimination of MPV, the basic-service husbandry option for mice was discontinued in the affected facility and replaced with the basic-plus husbandry option to minimize the risk of fomite-based contamination of mouse colonies with murine pathogens. Basic-plus husbandry includes the use of sanitized cage components, which are assembled with food and bedding materials, autoclaved at 220 °F (5 min sterilize, 15 min dry time), and stored as a complete cage unit until needed for routine cage changing and cage manipulations. However, water delivery continues to be by automatic watering. Water valves are autoclaved at 220 °F (10 min sterilize, 5 min dry time) prior to being installed on the racks. Once installed, water valves are either replaced or sprayed with 1:5:1 Clidox before docking a cage if the cage is being moved from its original location or a new cage is being docked into a slot with an existing water valve.

The following precautions were emphasized to the research community that housed mice in the affected facility through local postings, meetings, and electronic communications: 1) Water valves must be changed or sanitized (Clidox 1:5:1) prior to relocation of cages to avoid contamination of mice; 2) The opening of cages and manipulation of mice must take place within a biological safety cabinet located in the animal and procedure rooms; 3) Gloves, instruments, and interior hood surfaces should be decontaminated with chlorine dioxide (Clidox 1:5:1) before beginning work in the hood, between cages of mice, and after work is completed; 4) Mice must stay in their original rack locations once targeted testing has started; 5) Mice being weaned should remain within the same designated rows as the parent cage to ensure the progeny were under surveillance by the same targeted sentinel mice; 6) In addition to standard gloves and gown entry requirements, shoe covers must be put on upon entry and removed before stepping into the hall, and gowns must be changed upon exit from the room. Further, all proposed mice scheduled for relocation were tested by either serology or PCR, depending on their immune status, prior to relocation, regardless of room health status.

Sample collection. A single fecal pellet was collected from the anus of each unanaesthetized mouse while it was restrained gently. Fecal pellets from 2 or 3 mice in each cage were pooled and frozen at –70 °C pending PCR analysis. Blood samples of 100 to 200 µL were collected by retroorbital centesis by an experienced technician (with appropriate training and IACUC approval), or blood was collected by cardiocentesis after euthanasia by carbon dioxide overdose.

Serology and molecular assays. Sera were tested for MPV antibodies by using an immunofluorescent antibody assay as previously described.[15] Fecal pellets were homogenized in 400 µL PBS, and DNA was purified (DNeasy Tissue Kit, Qiagen, Valencia, CA) according to the manufacturer's instructions. PCR was performed (DyNAmo SYBR Green qPCR Kit, MJ

Research, Waltham, MA, or PCR Core Kit, Roche, Indianapolis, IN) by using primers specific for the MPV nonstructural gene.[4,7] Reaction conditions were: 2 min at 94 °C; 35 cycles of 30 s at 92 °C, 30 s at 50 °C, 60 s at 72 °C; and 5 min at 72 °C. PCR primers were obtained from the WM Keck Foundation Biotechnology Resource Laboratory at Yale University. All PCR assays included positive and negative controls.

Results

Targeted sentinels. Twelve rooms (containing 61 racks) successfully completed the targeted sentinel testing and cull process and are now considered cleared of MPV infection. Targeted sentinel results indicated that MPV was detected in 39 of 3584 targeted sentinel mice tested (1%). A subset of colony mice (representing 668 cages) housed within these positive rows were tested directly by serology, and 40 cages (6%) tested housed at least one MPV-seropositive mouse.

Overall, the number of seropositive routine soiled-bedding sentinels in each of the 12 rooms that completed targeted testing ranged from 1 to 4, with 4% to 25% (average, 11%) of routine sentinels in a room being seropositive. Three to 10 rounds of targeted sentinel testing were required before each room was determined to be cleared of MPV, as evidenced by 3 consecutive rounds of negative MPV serology results (Table 1). The mean duration of the targeted sentinel program (time from when the first set of targeted sentinels was placed until the room was deemed 'cleared with a history of MPV') was 20 wk.

In addition, 2 rooms were depopulated after 2 consecutive rounds of seropositive targeted sentinel results. One room was a 2-rack room with 2 rounds of MPV seropositive targeted sentinel results (1 of 18 and 3 of 18 positive), and the other was a 4-rack room with an initial negative targeted sentinel results that was followed by 2 consecutive rounds of seropositive targeted sentinels (1 of 30 and 3 of 30 positive). Because the majority of mice in these 2 rooms could be obtained from other sources, a decision was made to depopulate these rooms rather than to continue the targeted sentinel process.

Modified quarantine. The modified quarantine procedure was performed twice in mice housed in the same MPV-positive room. Each mouse in every cage (162 total mice housed among the 75 cages) was bled for MPV serology, and feces were collected for MPV PCR. Until testing was completed, cages were not moved, opened, or manipulated. Because all initial MPV serology and PCR results were negative, all 75 cages were relocated as a group to the adjacent empty animal room. The mice then were retested by serology 3 wk after the initial testing, with all MPV serology results again being negative. These mice remained in the new animal room and were used as breeding stock to populate the room. Another subset of animals was selected from the same rooms 6 mo later. The same procedure was repeated in 161 mice spread among 58 cages. The only difference was that these mice were first moved into an empty animal room just prior to the initial round of testing. As in the first case, all serology and PCR results for the 161 mice were negative for MPV. Therefore, a total of 323 mice in 133 cages were tested, released, and successfully used as core breeding mice to reestablish unique colonies of mice. Both new rooms repopulated by these mice have remained MPV-negative for more than 4.5 y, based on routine sentinel testing.

Postclearance events. Targeted sentinel testing was used to clear 12 MPV-contaminated rooms, and a modified quarantine process was used to test unique strains of mice to repopulate 2 new animal rooms. All of the targeted sentinel and modified quarantine testing was completed 4 to 5 y ago (depending on the initial date of detection and the duration of the infection within the room). The 12 rooms that underwent the targeted sentinel test-and-cull process have undergone an additional 190 sentinel screens, representing the serologic testing of 4102 sentinel mice, all of which have been negative for MPV (Tables 2 and 3). In addition, testing of investigator mice being relocated to other rooms during this same 4- to 5-y period resulted in MPV testing of an additional 3813 mice housed within the 12 affected rooms (Table 4). All of these investigator mice were seronegative for MPV. Although the investigator mice tested were not evenly distributed, either by room or by years, every room had multiple mice tested during at least 2 of the years. With one exception, the original populations in these rooms remained in the same room, and the rooms have housed mice continually since the infection. One room was decommissioned for a renovation, and mice were moved into a different room where testing continued and test data from the original room were merged with test data from the new room.

The additional 2 rooms that were populated with mice that underwent the modified quarantine testing have remained negative as well. These rooms have since undergone 16 and 22 rounds (representing 192 and 896 sentinel mice, respectively) of quarterly sentinel serology testing for MPV and have remained negative.

The fact that these 14 rooms have remained negative for MPV for 4 to 5 y, as well as the other 17 mouse rooms in the facility remaining MPV negative, suggests that the infection was eliminated and not simply driven to levels low enough to escape detection.

Discussion

One alternative to large-scale culling of mice to address an active MPV infection is the use of a targeted sentinel approach, a modification of the standard soiled bedding sentinel program, in which additional sentinels are used to sample an MPV-positive rack. This increased sentinel-to-cage ratio results in exposure of sentinels to soiled bedding from all the cages on a rack at a single time point. The efficiency of the targeted sentinel strategy lies in the ability to simultaneously sample all rows at the time of cage change, rather than consecutively. In addition, the additional sentinels allow the infection to be narrowed to 2 to 3 rows of racks during a 2-wk interval, whereas for standard bedding sentinels the location and timing of infection is much broader (one side of a rack during a 3-mo period). Although testing of all cages on a rack is expedited as compared with the routine method, the targeted sentinel method still requires several weeks to a few months to allow for seroconversion after infection of the targeted sentinels and to obtain repeated negative test results. Multiple rounds of serology testing were performed before a room was considered clear of MPV to compensate for the low infectivity of MPV and the fact that it can persist at a low prevalence in isolated pockets within a colony, thus making it difficult to detect. The decision to use 3 rounds of seronegative results as our standard for declaring a room cleared of MPV was somewhat arbitrary but was chosen to balance the relative difficulty of detection with the labor and cost involved in the testing process. We conclude that at least 3 consecutive negative rounds of targeted sentinel tests should be performed, because in 2 rooms yielded 2 consecutive negative rounds of serology testing for MPV followed by a positive round of serology testing.

Although the use of large volumes of soiled bedding are more effective at detecting MPV than are small volumes,[2,16] the targeted sentinel strategy used the standard 25 mL of bedding from

Table 1. Results from MPV room sentinels and targeted sentinel testing

MPV-positive room	no. of racks in room	no. of routine sentinels in room	no. of MPV seropositive routine sentinels in room	no. of rounds of targeted testing[a]
A	6	23	1	8
B	6	21	3	4
C	5	12	2	5
D	2	8	2	5
E	3	12	2	5
F	5	20	4	10
G	8	32	2	8
H	4	24	2	5
I	6	24	4	8
J	7	40	2	5
K	6	24	2	3[b]
L	3	12	2	3[b]
Mean	5.08	21.0	2.33	5.75
Total	61	252	28	69

[a]To be declared free of MPV, a room had to undergo 3 rounds of serology testing with all targeted sentinels being seronegative
[b]Targeted sentinels did not detect infection in these rooms

Table 2. Rounds of quarterly sentinel testing for MPV antibodies after rooms were declared clear of MPV

Room	2005[a]	2006	2007	2008	2009	2010[b]	Total
A	1	4	4	4	4	1	18
B	1	5	3	4	4	1	18
C	0	2	4	5	3	1	15
D	3	4	4	4	4	1	20
E	1	4	4	4	4	1	18
F	0	3	4	4	3	1	15
G	2	4	3	4	3	2	18
H	0	2	4	4	3	1	14
I	0	1	5	1	1	0	8
J	0	3	4	3	4	1	15
K	0	2	4	3	4	1	14
L	0	4	4	4	4	1	17
Total	8	38	47	44	41	12	190

[a]2005 is September through December
[b]2010 is January through April

Table 3. Number of sentinel mice tested for MPV antibodies after rooms were declared clear of MPV

Room	2005	2006	2007	2008	2009	2010	Total
A	24	98	96	96	99	19	432
B	24	97	70	101	119	31	442
C	0	45	96	89	68	28	326
D	32	85	110	105	112	28	472
E	24	78	79	52	80	28	341
F	0	79	93	97	83	28	380
G	45	82	52	95	71	53	398
H	0	11	96	126	92	28	353
I	0	13	47	11	8	0	79
J	0	77	96	70	103	28	374
K	0	48	91	68	86	24	317
L	0	48	44	46	39	11	188
Total	149	761	970	956	960	306	4102

each cage, the same volume as used in our standard bedding sentinel program. This volume was used so that the combined soiled bedding volume, from the 14 to 21 cages housed on the 2 or 3 rows of the rack, was less than 500 mL.

The accuracy of targeted sentinel testing requires that investigators and staff maintain cages on the same 2 or 3 rows of the rack that contain the targeted sentinel used to monitor these rows. This requirement can be challenging if, as in our case, breeding is not halted and weaning continues to occur. In this situation, cage slots must be left empty in the designated rows to provide space for cages of weanlings. As mentioned previously, although cessation of breeding in the rooms housing MPV-infected mice would likely have expedited eradication of MPV, we made the decision to allow breeding to continue in an effort to minimize the effect of this process on ongoing research. Therefore we have demonstrated that targeted sentinel testing can be an effective means of eliminating MPV from a mouse colony despite active breeding in the colony.

Strategies that sample the mice within an individual cage or the cage itself, rather than sampling the row, rely less on maintaining the cage location for extended period of times. However, these options are labor-intensive, costly, and have several drawbacks. Examples of these alternative methods include direct serology testing of individual mice, which is faster than targeted sentinels but is very labor-intensive and may miss active infections during their early phase because of the MPV's relatively long seroconversion interval. Direct testing of mice also may include PCR analysis of feces to detect real-time shedding. In fact, we have shown that testing of individual feces collected directly from each mouse is the most reliable method to detect active infection, as compared with sentinels methods, but is among the most labor-intensive and costly methods.[7] Although pooling of fecal pellets from soiled bedding is an alternative to collecting feces directly from the mouse, to reduce sample collection labor and testing costs, there are limitations on the number of feces that can be pooled. Removing only 5 to 10 fecal pellets of approximately 1000 fecal pellets in a cage may result in false-negative results due to sampling error if only one mouse

Table 4. Number of investigator mice tested for MPV antibodies after rooms were declared clear of MPV

Room	2005	2006	2007	2008	2009	2010	Total
A	0	31	87	47	91	112	368
B	0	33	64	63	151	5	316
C	0	0	17	23	13	40	93
D	0	0	70	70	244	19	403
E	0	0	40	0	2	0	42
F	0	17	57	14	169	31	288
G	2	14	61	20	24	28	149
H	0	10	23	2	37	6	78
I	0	5	3	0	0	0	8
J	4	2	4	26	105	0	141
K	0	10	19	0	111	7	147
L	112	573	184	0	739	172	1780
Total	118	695	629	265	1686	420	3813

in the cage is shedding MPV, because only MPV-negative feces might be selected from the cage or because the concentration of MPV in the sample pool might be diluted to an undetectable level. Sampling cages by swabbing the bottom of the soiled cage is another option, and we found that the sensitivity of this method was similar to that of fecal PCR.[7] Cage swabbing has a labor-saving advantage compared with fecal collection, because cages can be sampled quickly during the cage changing process. In theory, using an alternative strategy of environmental screening, such as screening rack exhaust filters from ventilated cage racks, would be beneficial since it is inexpensive and requires little labor. However, previous testing of exhaust filters yielded variable detection of MPV infection depending on the air-flow system and type of rack used.[4]

A challenge for any detection method is the low prevalence of MPV infection. To detect infection within a colony, the binomial distribution formula is often used to determine the required sample size that must be tested. If the prevalence is assumed to be 10%, which is among the lowest common standard used in the industry, 25 to 30 animals must be tested to achieve a 95% confidence rate for detecting infection.[14] However, MPV's prevalence in the current study was approximately 1% at the row level and 6% when individual animals on positive rows were tested. These prevalences are well below the often-assumed 10%. Many factors may have contributed to the low observed prevalence, including environmental (for example, HEPA-filtered individually ventilated caging; changing of cages in biological safety cabinets; autoclaving of cages, cage component, and food) and biological (for example, age and genotype of the mice) factors. Because the majority of mice in this facility were on a C57BL/6 background, which is known to be resistant to MPV infection,[3,5] the level and duration of MPV infection were probably less than they would have been if the mice were of a more susceptible strain, such as BALB or C3H.[3] The interplay of immunodeficiency and genetic background of the mice and how these relate to infection risk are unknown.

Several factors may have increased the duration of the MPV outbreak in this facility. To decrease the intrusion of the control methods on investigators, a decision was made to continue breeding and to permit the introduction of new mice into the facility. This decision resulted in the periodic introduction throughout the outbreak of naïve mice susceptible to MPV infection. The decision to allow breeding to continue was made because many of the mice housed in the facility are not commercially available; an extended cessation of breeding could have resulted in the loss of these lines of mice if all mice of the line became too old to breed. More than half of the mice in this facility were genetically engineered, and many were at least mildly immunocompromised. Therefore, prolonged shedding of MPV may have occurred, as has been documented in SCID mice,[2] in some of the mice in this facility.

MPV is a nonenveloped virus, and on the basis of studies of other rodent parvoviruses, it is assumed to be highly stable in the environment.[17] Given the environmental stability and initial concern about how the infection was being spread, we instituted the practice of autoclaving of cages, cage components, food, and bedding as a unit and storing the cage units assembled to decrease the risk of fomite-based transmission. The use of an automatic watering system did not prevent the elimination of MPV. Although water valves can serve as fomites, the risk of transmission by means of water valves can be mitigated by careful husbandry practices, including autoclaving water valves prior their installation on the racks and disinfection of water valves prior to placing new cages in a previously occupied position on the rack. Although fomite-based transmission probably occurs, we and others[3,16] have shown that cohoused pairs of sentinels exposed to the same soiled bedding or to the same experimentally infected mice do not uniformly seroconvert. In fact, one study demonstrated that 35% of the time, only 1 of 2 cohoused 4- to 6-wk-old Swiss Webster sentinel mice seroconverted after 3 wk of exposure to soiled bedding from experimentally infected mice.[16] This phenomenon suggests that there is a threshold level of MPV needed for infection and calls into questions the ability of fomites, which presumably would harbor much less virus than would soiled bedding, to reach such a threshold. In retrospect, we also recognized that the spread of the infection room-to-room was most likely due to the trafficking of animals. However, given the long-term success of this management strategy and the lack of data on fomite transmission, we have elected to continuing autoclaving caging and cage components as described earlier.

Despite the challenges of detecting and addressing MPV infections, we have shown that the targeted sentinel and modified quarantine strategies in conjunction with investigator education and careful husbandry practice are viable options to depopulating a room to eliminate the infection. The current results suggest that routine soiled-bedding sentinels are sensitive enough to detect the level of infection that poses a transmission risk. If routine sentinels were missing levels of virus sufficient to initiate infection, undetected infection would have been expected to amplify in the colony, especially given the continued addition of naïve mice into the colony through breeding and importation of mice, and MPV would have been detected during the past 4 to 5 y by standard soiled bedding sentinels, just as the original MPV outbreak was detected.

Our results suggest that elimination of MPV from a large colony of mice is feasible without costly culling, large-scale rederivation, halting of breeding, or disruption of research provided that some basic infection control practices are put into place. Our findings also suggest that the risk of transmission by fomites is manageable, even when presumably high-risk conditions (for example, animals manipulated daily by investigators, their staff, and students) prevail. This strategy should be considered as an alternative to more costly strategies to eliminate MPV or to benign neglect when resources are limited.

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