Comparative Medicine
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Vol 57, No 1
February 2007
Pages 74-81

Gender Influences Infectivity in C57BL/6 Mice Exposed to Mouse Minute Virus

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Two natural outbreaks of mouse minute virus (MMV) are described. Observations during management of the naturally infected colonies led to a study in which 4-wk-old C57BL/6NCr and C57BL/6Tac mice were inoculated oronasally with an immunosuppressive variant of MMV (MMVi), as were adult C57BL/6NCr lactating dams or their pups (age, 10 d). By day 28 postinoculation, 100% of the 4-wk-old male C57BL/6NCr and C57BL/6Tac mice, 56.2% of 4-wk-old C57BL/6NCr female and 62.5% of 4-wk-old C57BL/6Tac female mice, 100% of adult lactating C57BL/6NCr dams, and 100% of inoculated pups (10 d) had seroconverted. Serologically positive nursing dams did not infect their nursing pups. In contrast, when nursing pups were inoculated, 100% of their dams seroconverted by 28 d postinoculation. Only 1 of 4 facility sentinels (Tac:SW female mice) seroconverted to MMVi and none of the 4 research sentinels (Tac:SW female mice) seroconverted under a once-weekly bedding transfer program. Consequently, 4 new research Tac:SW sentinels of each gender (n = 8) were placed in known-positive cages at cage-change; 100% of the male mice but 0% of the females seroconverted by day 48. Study results suggest gender influences both infectivity and the ability to detect subclinical infections of MMVi. Other factors that may influence detection of MMV include mouse strain or stock, short shedding period, and prolonged time between cage changes. In light of the data from both the natural infections and the experimental cases, cessation of breeding likely will be beneficial when trying to eradicate this virus.

Abbreviations: DCT, Division of Cancer Treatment; ELISA, enzyme-linked immunosorbent assay; IVC, individually ventilated cages; MMV, mouse minute virus; MMVi, mouse minute virus (immunosuppressive); MPV, mouse parvovirus; PCR, polymerase chain reaction

Mouse minute virus (MMV), also known as minute virus of mice, is a small (diameter, 15 to 28 nm) nonenveloped, single-stranded DNA virus of the Parvoviridae family.[17] Parvoviruses occur as natural infections in mice, are prevalent in research colonies, and infect a wide variety of hosts, ranging from insects to primates.[11,13,16,17,22] Historically high, the prevalence of MMV appears to be declining with improvements in husbandry practices.[16,24] Natural infections of mouse parvovirus (MPV) and MMV are often asymptomatic and apathogenic, even for neonatal and immunocompromised animals.[19] MMV causes an acute, selflimiting infection, with infant mice more susceptible to infection than adult mice.[9] In immunocompetent mice, viral replication occurs in the small intestine, liver, and lymphoid organs.[4,5] However, humoral immunity to either MPV or MMV is not cross-protective.[9] Transmission occurs by oronasal exposure. The virus can infect the gastrointestinal tract and is excreted in feces and urine. Transmission, therefore, is primarily direct, although more transmission studies are needed.

Parvoviruses generally are resistant to physiochemical treatments, such as heat, solvents, pH, and denaturing agents.[3] The resistance of rodent parvoviruses to environmental inactivation increases the risk of transmission after virus is excreted.[9] Therefore, potential contamination of caging, bedding, food, and clothing must be considered a risk for the spread of infection. Viral contamination of biologicals used for experimental inoculation, such as transplantable tumors, also can be a source of infection.[6] In addition, mouse strain and age have important roles in the seroconversion of parvoviruses in mouse tissues, therefore diagnostic serologic testing and polymerase chain reaction (PCR) analysis should be considered within the context of the mouse strain and age, especially when sentinel mice are used for surveillance.[4,5]

Review of the literature indicates that MMV appears to be a selflimiting infection that does not persist in immunocompetent mice.[9] Infection appears to last at least 3 wk in oronasally inoculated immunocompetent neonatal mice.[18] Elimination and control of the virus within a colony should combine the principles of quarantine, rigorous barrier husbandry procedures, regular surveillance of mice and mouse products destined for use in vivo, and cessation of breeding for 6 to 8 wk.

Two separate facilites at our institute experienced MMV outbreaks over a 4-y period. Both outbreaks were managed similarly to contain the infection, identify positive animals, and prevent reoccurrence of further outbreaks. Review of the sentinel program and serology test results from these 2 natural MMV outbreaks raised several questions. How long is MMV shed in the feces after an initial outbreak? Can natural infection with MMV be eliminated from a mouse colony with cessation of breeding? How sensitive and effective is our sentinel program in detecting a MMV outbreak?

The purpose of this study was to expand our knowledge of MMV shedding and transmission in a controlled breeding colony setting. Extrapolation of the study results may provide veterinarians with greater knowledge regarding managing a MMV outbreak without depopulating or rederiving an entire mouse colony. In addition, we describe the information needed to assess the effectiveness of a sentinel surveillance program.

Received: 2 Nov 2006. Revision requested: 25 Nov 2006. Accepted: 7 Dec 2006.
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History of 2 MMV Outbreaks at Our Institution

Facility I consists of 52 small mouse rooms containing a total of 11,000 static Micro-Isolator™ cages (Lab Products, Seaford, DE) with an average daily census of 33,000 mice. Cage units were changed twice a week. Personnel wore disposable jumpsuits, shoe covers, hair bonnets, facemasks, and gloves. Animals were housed in Micro-Isolator™ cages (Lab Products), which were changed with use of microisolator techniques in biosafety cabinets. After identification of the MMV outbreak, people entering the room wore an additional disposable jumpsuit (which was removed prior to exiting the animal room) and additional sets of shoe covers, hair bonnets, facemasks, and gloves.

MMV antibodies were first detected in a sentinel mouse in 1 room within this large rodent facility, which had been pathogen-free for several years. The MMV-postive sentinel was a Swiss Webster female mouse obtained from Taconic (Germantown, NY). Sentinels were routinely sent for diagnostic workup to screen for pathogenic agents every 6 wk. When MMV was detected, this room was placed under quarantine, and all cages, bedding, and caging supplies were bagged and autoclaved out. The remaining sentinels in the facility were sent to pathology for terminal bleeds, and new sentinels were ordered for the entire facility. Follow-up bleeding for MMV was performed on 1 mouse in each study cage in the first positive room within a few days of detection of the first positive sentinel. MMV serology testing then was performed in an adjoining room because of transfer of mice from the positive room prior to detecting MMV in the facility. Seropostive adults, nursing pups, and sentinels were submitted for PCR analysis.

Facility II consists of 2 rat rooms and 8 mouse rooms. Mice were housed in individually ventilated cages (IVC) on 126-cage racks, for a total of 2646 IVC cages with an average daily census of 10,680. Cage units were changed twice a week on open-top changing tables. Personnel wore disposable lab coats, shoe covers, and gloves. After identification of the MMV outbreak, additional personal protective equipment required were disposable jumpsuits over lab coats and additional sets of shoe covers, hair bonnets, facemasks, and gloves. The outer disposable jumpsuit was removed prior to exiting the animal room. Sentinel animals first tested positive for MMV antibodies in April 2003, at which time seroconversion was then detected in animals from 4 rooms on 6 different racks. Infection was confirmed by testing sera and tissues from cagemates of the index sentinels. Seroconversion was not detected in all cagemates of the index sentinels. A small group of mice were submitted for PCR analysis.

Infection Study
Materials and Methods

Animals. This study was reviewed and approved by the National Institute of Diabetes and Digestive and Kidney Diseases's Animal Care and Use Committee. All procedures and use of animals were in compliance with the *Guide for the Care and Use of Laboratory Animals*.[14] We obtained 4-wk-old female C57BL/6NCr and male C57BL/6NCr mice and adult C57BL/6NCr nursing dams with litters from the Division of Cancer Treatment (DCT; Frederick, MD). We also obtained 4-wk-old female and male C57BL/6NTac mice and 4-wk-old Tac:SW male and female mice (Taconic, Germantown, NY). All mice were received and maintained in a National Institutes of Health animal facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International. The mice were maintained in a quarantine room and were seronegative for CAR bacillus, ectromelia virus, epizootic diarrhea of infant mice virus, mouse encephalomyelitis virus, lymphocytic choriomeningitis virus, mouse cytomegalovirus, mouse hepatitis virus, mouse sialodacryoadenitis virus, MMV, *Mycobacterium* spp, MPV, pneumonia virus of mice, reovirus 3, and Sendai virus on arrival and were free of bacterial and parasitic infections. Mice were housed in 7.5 × 11.5 × 5-in. sterilized ventilated Micro-Isolator™ cages (Lab Products) on 1/8-in. bedding (Bed-o'cobs, Anderson, Maumee, OH). Cages were changed weekly, except for a subset of cages (n = 4) containing research mice that were changed every third day until 12 d and then weekly thereafter. The animal holding room was maintained under environmental conditions of 20 °C, 40% to 70% relative humidity, 15 air changes hourly, and a 12:12-h light: dark cycle. Mice were fed (Autoclavable Mouse and Rat Open Formula NIH-31 Diet, Zeigler Brothers, Gardners, PA) ad libitum and provided sterilized individual water bottles as an ad libitum water source. On arrival, the mice were acclimated for a minimum of 4 d before being used in experiments. Mice were identified with numbered stainless-steel rodent ear-tags (National Band and Tag, Newport, KY).

Infectious agent. MMVi virus obtained from the University of Missouri-Columbia was received frozen and held in a –80 °C freezer until time of usage, when it was placed in a –20 °C freezer overnight and then transferred to a 4 °C freezer and held at this temperature until ready to dilute. The stock (original concentration, 2×10^9 plaque-forming units/ml) was serially diluted (50 mM Tris base, 10 mM ethylenediaminetetraacetic acid; pH 8.7) to a final concentration of 1×10^4 plaque-forming units per milliliter.

Inoculations. Unanesthetized mice were experimentally infected oronasally with 5 µl MMVi virus, containing 5×10^4 median mouse infectious doses (5×10^4 plaque-forming units), as used in a previous study.[13]

Serology. Initial sentinel serology (index cases) for the natural infection was performed at BioReliance (Rockville, MD) for both Facility I and Facility II. For Facility II, serum samples were tested by enzyme-linked immunofluorescent assay (ELISA; rNS1) at the University of Missouri-Columbia Research Animal Diagnostic Laboratories, and those positive to MMV were confirmed by hemagglutination-inhibition assay and fluorogenic nuclease PCR[15] of mesenteric lymph nodes, kidney, and intestine. Subsequent to confirmation, all additional serology from sentinels and cull animals was performed at the University of Missouri-Columbia Research Animal Diagnostic Laboratories, as were ELISA serology tests on all research animals.

PCR amplification. PCR assays using previously published MMV-specific primer sequences[13] were performed by the Diagnostic Services of the Division of Veterinary Resources (National Institutes of Health, Bethesda, MD).

Study design. ***Group I.*** C57BL/6NCr mice were allocated randomly 4 to a cage according to sex (4 cages per sex). The mice were ear-tagged and given the infective agent oronasally. Blood samples collected by tail nick or submandibular bleed were taken at 3, 8, 10, 13, 16, and 28 d postinoculation. Pooled fecal samples were obtained from each cage for MMV PCR on days 8, 12, 21 and 28. The experiment was repeated using 2 cages of each sex of C57BL/6NTac mice. Blood samples were taken by submandibular bleed at 2, 9, 13, 16, and 28 d postinoculation. Pooled fecal PCR assays for MMV were performed on days 2, 5, 7, 9 and 12 postinoculation.

Group II. Four adult lactating C57BL/6NCr mice were inoculated oronasally as described previously; pups (n = 21) were not inoculated. Serology samples for MMV were taken by submandibular bleed from the dams on days 3, 10, 16, and 28 postinoculation. Live pups (n = 16) were submitted to the diagnostic laboratory and euthanized with compressed carbon dioxide gas for sample collection on days 8, 14, and 28. Blood samples were obtained for serology, and mesenteric lymph nodes, kidney, and intestine for MMV PCR. Remaining pups were weaned at 21 d of age, placed with animals of the same sex, and ear-tagged. Fecal samples from each cage containing pups were sent for MMV PCR on days 8, 14, and 21 postinoculation.

Group III. In 4 cages of lactating C57BL/6NCr mice, all pups (n = 20) were inoculated oronasally at 10 d of age; their dams were not inoculated. Serology samples for MMV were taken by submandibular bleed from the dams on days 3, 10, 16, and 28. Pups (n = 16) were submitted to pathology and euthanized with compressed carbon dioxide gas. Samples were obtained for serology and for MMV PCR of mesenteric lymph nodes, kidney, intestine and fecal samples on days 8, 14, and 28. Pooled fecal samples from each cage were sent for MMV PCR on days 8, 14, and 21 postinoculation. Remaining pups were weaned at 21 d of age, placed with animals of the same sex, and ear-tagged.

Group IV. Eight serologically MMV-positive dams from groups II and III were mated with seropositive males from group I at 6 wk after initial inoculations. Blood samples were taken from individual pups (n = 41) on day 23, 42, 58, or 76 after birth. The pairs were allowed to continue to breed; random pups from their second and third litters were euthanized with compressed carbon dioxide gas on days 26 to 58 after birth and tissue samples tested by MMV PCR.

Group V—sentinel monitoring program. Three groups of Tac:SW mice (Germantown, NY) were used as sentinels. Sentinel group A (n = 3, female mice) comprised the 'facility rack sentinels,' which were supplied and used by the facility in which the animals were housed. Sentinel groups B (n = 3, female mice) and C (n = 8, 4 male and 4 female) were the 'research sentinels,' which were used specifically for this research project.

Approximately 1 tablespoon (12.3 g) of dirty bedding was taken once weekly during cage changes from all cages housing research mice in groups I through IV and placed in the Sentinel group A (facility sentinels) cage and Sentinel group B (research sentinels) cages. Sentinel group C mice were placed directly in cages containing a mixture of dirty bedding from group I after cage changes 3 times weekly for 12 d. Blood samples were collected by submandibular bleed from the sentinel animals on days 0, 8, 14, 16, 28, 43, 48, 51, 91, 115, 126, 141, and 170 and submitted for MMV serology. Pooled fecal samples underwent MMV PCR on days 8, 14, 21, and 28 for the group B research sentinels (female mice).

Results

Natural infection. In facility I, 28 of 474 cages of research mice in 2 animal rooms were identified as seropositive for MMV after a seropositive sentinel was found in August 1999. Mice in seropositive cages were euthanized with carbon dioxide gas. Remaining research and sentinel mice were tested every 4 wk. At 4 wk, 6 additional study mice from the first animal room had seroconverted to MMV; sentinel mice in this room continued to test negative for MMV. Sentinels, seropositive adults, and nursing pups were negative for MMV by PCR assay. Results from other sentinels in facility I (samples were submitted 2 wk after the first positive case) revealed that 5 additional rooms on 3 separate floors were positive for MMV. These rooms were quarantined and handled in the same way as the first positive rooms. Breeding was allowed to continue in most rooms, and seropositive mice and cagemates were removed, bled, and euthanized every 4 wk. In addition, all offspring weaned from seropositive cages were euthanized. One MMV-seropositive room was the transgenic core facility; this room was depopulated. The index rooms were negative for MMV within 2 mo. The entire facility was negative for MMV within 4 mo and has been negative for MMV for the last 6 y.

In facility II, sentinel mice first tested positive for MMV antibodies in April 2003, at which time seroconversion was detected in sentinel animals from 4 rooms on 6 different racks. Infection was confirmed by testing sera and tissues from cagemates of the index sentinels. Seroconversion was not detected in all cagemates of the index sentinels. PCR analysis[15] revealed the presence of virus in mesenteric lymph nodes of a small group of mice (approximately 1%). Seroconversion subsequently was detected in colony animals as well.

A variety of control measures were instituted. Investigators were asked to cull all nonessential animals from positive racks. Additional personal protective equipment was required in positive rooms and was discarded on exit from the rooms. Wherever possible, breeding was halted, and animal orders were stopped. Animal movement between rooms was prohibited, and attempts were made to limit movement of cages between racks within rooms. Serum from 1 animal from each cage of euthanized animals throughout the facility was submitted for testing; 1 mouse from each cage on positive racks in rooms under quarantine was bled monthly. All positive test results were traced back, and animals known to have contact with positive animals were euthanized. Caging from affected areas was placed in autoclavable plastic bags within the rooms. The outsides of the bags were sprayed with chlorine dioxide before exiting the room. The bags then were transported to the dirty cagewash area and autoclaved prior to bedding disposal and sanitation.

Despite these measures, evidence of infection eventually involved a total of 18 racks in 5 animal rooms with 42 positive mice out of a population of 10,680 mice. The criteria for declaring a room free of infection were negative serologic results from all cages culled and from all animals tested during survey bleeding from that room for 2 consecutive months. Rooms in which all control measures were instituted were declared free of infection 4 mo later. The entire facility was declared free of infection, 8 mo after the initial positive result. Subsequently, cull animals from this facility continued to be tested as part of the sentinel program. All samples have been negative for antibodies to MMV for the last 3 y.

Infection study. ***Group I (male mice).*** The C57BL/6NCr male mice were serologically negative for MMV on days 3 and 8. On day 10, 12.5% of mice (2 of 16) were positive for MMV; on day 13, 68.7% (11 of 16) were positive; on day 16, 93.75% (15 of 16) were positive; and on day 28, 100% (16 of 16) of these mice were serologically positive for MMV. Similarly, with the C57BL/6NTac mice in group I, male mice (n = 8) were serologically negative for MMV on days 2, 9, and 13. On day 16, 37.5% (3 of 8 mice) were positive for MMV, and on day 28, 100% (8 of 8) were serologically positive (Table 1).

Group I (female mice). Female C57BL/6NCr mice were serologically negative for MMV on days 3 and 8. On days 10 and 13,

Table 1. MMV ELISA serology results

Mice	No. seropositive/no. tested on postinoculation day									
	2	3	8	9	10	13	14	16	28	51
Group I: 4-wk-old MMV-inoculated mice										
C57BL/6NCr										
Male	–[a]	0/4	0/16	–	2/16	11/16	–	15/16	16/16	–
Female	–	0/4	0/16	–	1/16	1/16	–	4/16	9/16	–
C57B16/NTac										
Male	0/2	–	–	0/8	–	0/8	–	3/8	8/8	–
Female	0/2	–	–	0/8	–	1/8	–	1/8	5/8	–
Total males from both vendors	0/2	0/4	0/16	0/8	2/16	11/24	–	18/24	24/24	–
Total females from both vendors	0/2	0/4	0/16	0/8	1/16	2/24	–	5/24	14/24	–
Group II: MMV- inoculated C57BL/6NCr dams with naïve pups										
Dams	–	0/4	–	–	0/4	–	–	2/4	4/4	–
Pups (either sex)	–	–	0/6	–	–	–	0/6	–	0/4	0/4
Group III: MMV-inoculated C57BL/6NCr pups with naïve dams										
Dams	–	0/4	–	–	0/4	–	–	1/4	4/4	–
Pups (either sex)	–	–	0/6	–	–	–	5/6	–	4/4	–
	Days postpartum									
	23	26	42	47	50	58	76			
Group IV: Pups from MMV-positive breeders										
Litter 1	0/4	–	0/3	–	–	0/2	0/4			
Litter 2	–	0/6	–	0/2	–	–	–			
Litter 3	–	0/6	–	–	0/7	0/7	–			

[a]No samples taken.

6.2% (1 of 16) mice were seropositive for MMV. On day 16, 25% (4 of 16) tested positive for MMV, and on day 28, 56.2% (9 of 16) were seropositive for MMV. Among female C57BL/6NTac mice, on days 2 and 9, all 10 samples submitted were negative, and on days 13 and 16, 12.5% of samples (1 of 8) were positive on each day. On day 28, 62.5% of samples (5 of 8) were seropositive for MMV (Table 1).

Pooled fecal PCR results for group I C57BL/6NCr male mice were all negative for MMV on days 8, 12, 21, and 28. For the group I C57BL/6NTac male mice, 1 (n = 4 mice) of 2 cages was PCR-positive for MMV on day 9; all other pooled fecal samples (days 2, 5, 7, and 12) were PCR-negative (Table 2). All pooled fecal PCR results for both C57BL/6NCr and C57BL/6NTac female mice were negative for MMV on days 2, 5, 7, 8, 9, 12, 21, and 28 (Table 2).

Group II (inoculated lactating C57BL/6NCr dams). Blood samples from the inoculated dams were seronegative on days 3 and 10, but on day 16, 50% (2 of 4) were positive, and on day 28, all dams (4 of 4) had seroconverted to MMV. On days 8, 14, 28, and 51, blood samples from a random selection of pups from each cage with inoculated dams were seronegative for MMV (Table 1). PCR assays performed on mesenteric lymph node, kidney, intestine, and fecal samples of pups in group II on days 8, 14, and 28 were all negative for MMV. (Table 2)

Group III (naïve lactating C57BL/6NCr dams). The 4 dams in this group were seronegative for MMV on both days 3 and 10, but 25% (1 of 4) were seropositive on day 16, and 100% (4 of 4) were seropositive to MMV on day 28 (Table 1). On day 8, all 6 inoculated pups were negative for MMV, but on day 14, 83.3% (5 of 6) were seropositive for MMV, and on day 28, all 4 pups tested were positive. On day 8, 50% (3 of 6) of the mesenteric lymph node samples and 83.3% (5 of 6) of the intestinal tissue samples were positive for MMV by PCR assay. All other samples tested by PCR on days 8, 14, 21, and 28 were negative for MMV (Table 2).

Group IV (MMV-seropositive breeding pairs). Parents were retested serologically for MMV on the day that they were paired, and all 16 remained seropositive to MMV. Pups from the first litter of each pair were selected randomly, euthanized, and tested; none of the animals tested on days 23 (n = 4), 42 (n = 3), 58 (n = 2), or day 76 (n = 4) were seropositive for MMV. Random pups also were selected from the second and third litters of seropositive pairs and tested for MMV. All pups were seronegative for MMV on all test days (Table 1).

Group V. Research and facility sentinels (Tac:SW) were tested for MMV serologically and by PCR after experimental inoculation of research mice. Female facility sentinels for the quarantine facility tested negative for MMV on days 43, 91, and 141; a single facility sentinel tested positive on day 115. The research sentinels (group B), which were female mice, tested negative for MMV on all time points (days 0, 8, 14, 16, 28, 48, 51, 126, and 170). The group C research sentinels (male and female mice) were exposed directly to dirty cages and then tested for MMV. All 4 (100%) of the male mice tested positive for MMV on days 48 and 170, whereas all female research sentinels were seronegative to MMV

Table 2. Results for MMV PCR

Mice	No. positive/no. tested on postinoculation day								
	2	5	7	8	9	12	14	21	28
Group I: Pooled fecal sample									
C57BL/6NCr									
Male	–[a]	–	–	0/4	–	0/4	–	0/4	0/7
Female	–	–	–	0/4	–	0/4	–	0/4	0/7
C57BL/6NTac									
Male	0/2	0/2	0/2	–	1/2	0/2	–	–	–
Female	0/2	0/2	0/2	–	0/2	0/2	–	–	–
Group II: C57BL/6NCr pups (dam inoculated)									
Mesenteric lymph node	–	–	–	0/6	–	–	0/4	–	0/4
Kidney	–	–	–	0/6	–	–	0/4	–	0/4
Intestine	–	–	–	0/6	–	–	0/4	–	0/4
Feces	–	–	–	0/6	–	–	0/4	0/4	0/4
Group III: C57BL/6NCr pups (pups inoculated)									
Mesenteric lymph node	–	–	–	3/6	–	–	0/4	–	0/4
Kidney	–	–	–	0/6	–	–	0/4	–	0/4
Intestine	–	–	–	5/6	–	–	0/4	–	0/4
Feces	–	–	–	0/6	–	–	0/4	0/8	0/4
Group V: Tac:SW research sentinels									
Female (pooled fecal sample)	–	–	–	0/2	–	–	0/1	0/2	0/2

[a]No samples taken.

at these time points (Table 3). Pooled fecal samples taken on day 8, 14, 21, and 28 from the female research sentinels were negative by MMV PCR (Table 2).

Discussion

Two separate MMV outbreaks occurred in 2 large facilities at our institute over a 4-y period. Facility I housed mice in microisolator cages, and facility II housed mice in ventilated cages. Cages were changed twice weekly in both facilities, and MMV was detected during testing of the sentinel mice. Although the source of the virus remains unknown, both facilities successfully eliminated the infection by testing 1 mouse from each cage and cages containing culling seropositive mice.

PCR techniques were used during both outbreaks for detection of active shedding of MMV and for identifying MMV DNA in mesenteric lymph node, kidney, and intestinal tissues and fecal pellets. PCR assays (data not shown) of samples from facility II were helpful in identifying positive animals from positive rooms, but harvesting mesenteric lymph nodes from all culled mice is labor-intensive. Although fecal PCR assays are most effective during or close to the acute phase of infection, selecting mice that are shedding virus is difficult, because MMV infections are generally subclinical in immunocompetent mice.[1] Because of the large number of animals in the facilities, we did not find that PCR testing of sentinels and colony-culled animals was cost-effective during these 2 outbreaks.

In facility I, we noted seroconversion of male pups to MMV but female littermates were seronegative during the outbreaks, even though both the sire and dam of the litter were seropositive for MMV. In addition, all pups from the second litters of MMV-positive breeding pairs were positive for MMV. We speculate that the infection occurred close to the time of weaning of the first litter and that the second litter was infected the first few days after birth; therefore both male and female pups seroconverted. These findings indicated to us that not only age, but gender, may influence the susceptibility of mice to infection with MMV.

Although C57BL/6 mice are resistant to clinical signs of MMV,[5] we selected this strain for experimental inoculations because a large proportion (>90%) of our research mice have a C57BL/6 background. In the experimental study, oronasal inoculation of C57BL/6 mice with MMV resulted in seroconversion of all adult males, compared with only 58% of the female C57BL/6 mice. These findings suggest that C57BL/6 female mice are more resistant to MMV infection than are C57BL/6 males, similar to observations during the natural MMV infections at our facilities. These differences found may have been due to the different genetic backgrounds between the mice we used in these studies, the different pathogen status of the animals, or even possibly the result of pooling of fecal samples, which could lead to false negatives due to a dilution effect from the MMV-negative animals in the cage. Our C57BL/6 MMV-positive mice shed MMV virus in their feces for only 9 to 12 d, which is shorter than the shedding time reported for a natural infection.[1]

Inoculated dams were seropositive at day 16 postinoculation whereas inoculated pups were seropositive on day 14. No nursing pups seroconverted when only the dams were inoculated (Table 1, group II). In contrast, all dams of inoculated pups seroconverted (Table 1, group III), and all inoculated pups were seropositive to MMV by at least day 28 postinoculation. By day 28, 100% of the dams (both groups) had seroconverted. The noninoculated dams probably ingested virus while cleaning and grooming the inoculated nursing pups. Parvoviruses are highly dependent on host cell function for replication and are transiently expressed during the S-phase of the cell cycle, limiting parvovirus replication to mitotically active cells.[5] We speculate that the pups were heavily infected due to their numerous rapidly dividing cells (growth) and

Table 3. MMV ELISA serology results for Tac:SW sentinels (group V)

Mice	No. positive/ no. tested on postinoculation day												
	0	8	14	16	28	43	48	51	91	115	126	141	170
Facility sentinels													
Tac:SW females													
Group A	–[a]	–	–	–	–	0/1	–	–	0/1	1/1	–	0/1	–
1 cage change/wk													
Research sentinels													
Tac:SW females													
Group B	0/2	0/2	0/1	0/3	0/3	–	0/4	0/2	–	–	0/3	–	0/4
1 cage change/wk													
Group C	–	–	–	–	–	–	0/4	–	–	–	–	–	0/4
3 cage changes/wk													
Tac:SW males								–	–	–	–	–	
Group C	–	–	–	–	–	–	4/4	–	–	–	–	–	4/4
3 cage changes/wk													

[a]No samples taken.

thus were shedding large numbers of viral particles. This heavy viral shedding by the pups potentially resulted in seroconversion of the dams (due to hygiene of the young and coprophagy).

In contrast, when their dams were inoculated, pups did not seroconvert. We speculate that this finding reflects the fewer mitotically active cells relative to the body size of the dams and therefore a low infectious burden. We suspect that inoculated dams did not shed virus particles in sufficient concentration to constitute an 'infective dose' even for pups, which arguably are at increased risk due to their large number of mitotically active cells. We theorize that positive dams do not transmit MMV to their pups because of maternal antibody protection, combined with the less intense shedding of viral particles from dams due to their fewer mitotically active cells for parvovirus replication. In addition, pups are not coprophagic until day 14 postpartum and therefore, considering the brief duration of viral shedding in the feces, pups have limited opportunity to ingest the virus. The breeding study (group IV) suggests that cessation of breeding may help to eliminate MMV from colony animals during an outbreak. Pups born from seropositive parents did not seroconvert (Table 1, group IV).

No research sentinels seroconverted before day 48 (Table 3), and these were male mice that were transferred directly to dirty cages from previously identified seropositive mice. These results are similar to those found in a previous study using MPV.[2] As in the previous study,[2] animals with low infective doses (that is, 5×10^4 virions[13] or less) were not detected via ELISA or PCR assay. Positive findings were detected on indirect fluorescent antibody, hemagglutination-inhibition, and PCR assays only after a 10-fold higher dose of MPV viral inoculum was given.[2] The results of the previous study[2] also showed that mouse strain and age affects seroconversion to MPV. We suggest that our sentinel animals were possibly exposed to only a low infective dose of MMV in the dirty bedding. Although the female research sentinel mice did not seroconvert to MMV, perhaps due to low exposure or greater resistance to MMV, the male research sentinel mice did seroconvert. Time to seroconversion to MMV is an important factor in structuring a sentinel program. Several recent articles address the inadequacy of current sentinel programs with respect to efficient detection of MPV.[7,8,20,23] Preliminary results from our ongoing studies indicate that gender, age, and dose all influence infectivity and shedding of MMV in C57BL/6 mice and suggest that female C57BL/6 mice may require a higher inoculation dose than do males to seroconvert to MMV (unpublished data).[21] Further work must be completed to determine the infectious doses of MMV in male and female mice.

Our present study indicates that MMV is self-limiting and that cessation of breeding combined with testing and removing of positive cages can eliminate the virus. We recommend cessation of breeding for 6 to 8 wk in order to 'burn out' the virus. Prior to reinitiating breeding, we suggest testing breeder pairs by both serology and fecal PCR to identify transgenic mice that may still be shedding MMV due to unknown immunodeficiency status. Fecal PCR for MMV could be used as an adjunct testing method in animals prior to seroconversion or in immunocompromised animals. We recommend that investigators assist in the management of their breeding programs and with the animal health sentinel programs, both of which would help in preventing infections and potential spread of infectious agents. Litters should not be mixed at weaning, as this practice, usually performed to save cage space, risks the introduction of disease from infected to naïve litters. Combining litters may allow new infections and increase the duration of viral shedding in the colony.

Female research sentinel mice were seronegative to MMV at most time points. This finding suggests that because we use female SW mice, our current sentinel program maybe ineffective in detecting MMV. Current laboratory practices recommend the use of female mice as sentinels to minimize behavioral problems seen with male group-housed mice (aggression).

To determine whether male sentinels would seroconvert, we used Tac:SW male mice as sentinels. The research sentinel Tac:SW males seroconverted to MMV after transfer of dirty bedding 3 times weekly for 12 d, whereas the female Tac:SW research sentinels were negative to MMV after direct transfer to dirty bedding both once and 3 times weekly. In other words, direct transfer of SW sentinel mice to known-positive dirty cages resulted in seroconversion to MMV in male, but not female, Tac:SW sentinel mice. These results are similar to those demonstrating lack of transfer of MPV1a after the transfer of dirty bedding to sentinel cages housing female Tac:SW mice.[7] Due to increased use of IVC, once-a-week cage changes, and the short shedding period of MMV, transmission and detection of virus may be limited. However, we

showed that by directly adding mice to dirty cages during experimental inoculation, Tac:SW sentinel male, but not female, mice seroconverted to MMVi 100% of the time. Different results could occur during natural infections if low amounts of virus are shed.

We suggest that current sentinel methods must be challenged or modified when testing for parvoviruses. We recommend consideration of sensitive strains[2,4] of male sentinel animals if detection of parvoviridae is essential. Group-housing male sentinel mice may lead to fighting and other dominance behaviors, which may require more clinical treatment or culling of mice. Accommodating these behavioral issues may require more cage space for separation of male mice, thereby increasing costs for treatment and equipment, increasing direct costs to investigators and institutes, and increasing the overall cost of research. Our data suggest that sentinel programs may not detect MMV (or Parvoviridae, in general) infection in mouse colonies, particularly as MPV is inefficiently transmitted with soiled bedding.[7] The efficacy of MMV transmission to our sentinel mice depended on several factors, many of which also affect transmission of MPV.[7]

Smith and Compton have suggested continuous reassessment of surveillance programs.[20] Three common strategies for a sentinel program include selecting sentinels directly from research animals, housing sentinels in direct contact with research animals, and transfer of soiled bedding. Soiled bedding transfer is perhaps the most common method, but standard operating procedures for collecting soiled bedding vary among animal facilities. The quantity of bedding collected, number of cages sampled, and frequency of collection are all variables that may influence the likelihood of detecting various pathogens. For each pathogen to be excluded, it is important to know: 1) the infectious dose required for seroconversion; 2) the window and route of shedding; 3) age and immune status of host animals; and 4) colony turnover. Compton and colleagues[7] suggested that IVC cages present new challenges for surveillance. Transferring a representative sample of dirty bedding from each cage on a rack to a sentinel cage at cage-change times likely will detect infectious agents that pass through the gastrointestinal and genitourinary systems but not the respiratory system.[7] Their results showed that although direct-contact sentinels detected all agents, Sendai virus was detected only in air samples, whereas MPV and *Helicobacter* were detected by use of soiled bedding, for which the efficacy of detection was dependent on the frequency and dilution of the soiled bedding transferred. The use of static or IVC cages did not influence detection rates. The investigators recommended a multifaceted approach involving a combination of detection systems,[7] thus potentially increasing costs. Another possible modification for detection of outbreaks would be to rotate sentinel mice through dirty cages in addition to adding dirty bedding.

In conclusion, we show here that the rate of infection of C57BL/6 mice with MMVi differs according to gender. Combined with testing and removal of positive cages, we recommend cessation of breeding for 6 to 8 wk in MMV-infected colonies, which may provide an extra safeguard to the elimination of MMV in an animal facility. In addition, female SW sentinel mice used for surveillance do not seroconvert as readily as do male mice using a system of exposing sentinel mice to soiled bedding during once-weekly cage change of the rack. This study raises questions regarding the true prevalence of subclinical MMV infection in mice, as many sentinel programs use female mice, cages are changed weekly or even less frequently (IVC), and the phase of MMV shedding is short. Several caveats apply to our experimental findings regarding the critical window of shedding and gender- and age-associated sensitivity to MMV. First, C57BL/6 mice are considered fairly resistant to parvoviruses.[5] More sensitive strains or more hardy stocks of mice may differ significantly regarding the period of shedding of the virus. Second, in parvovirus-sensitive strains of mice, gender may not influence susceptibility as strongly as in C57BL/6 mice. For example, female mice of sensitive strains may seroconvert at a later date than do males, or gender may have no influence. Third, sensitive strains may shed greater numbers of viral particles, which could affect the infection rate of pups from infected dams. Future studies using different strains or stocks of mice could determine which are better suited for use as sentinels.

Acknowledgments

We would like to thank Myrna Mandel, PhD, for her extraordinary work on all the PCR sampling, the dedicated care staff of the Division of Veterinary Resources (especially Devorah Crown), and the guidance and assistance of Dale A Smith, PhD (Uniformed Services University of Health Sciences, Bethesda, MD). This research was supported by the Intramural Research Program at the National Institutes of Health (Division of Veterinary Resources, Office of Research Services, and National Institute of Diabetes and Digestive and Kidney Diseases).

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