Journal of the American Association for Laboratory Animal Science
Vol 47, No 1
January 2008
Pages 26–30
Copyright 2008
by the American Association for Laboratory Animal Science

Eradication of Murine Norovirus from a Mouse Barrier Facility

Robin J Kastenmayer,* Kathy A Perdue, and William R Elkins

Murine norovirus (MNV) is a common viral infection of mice in many research facilities. MNV infects hematopoietic cells and alters their cellular morphology. Because of MNV's probable effects on the systemic immune response of infected mice the decision was made to eradicate the virus from 2 rooms containing infected animals in our vivarium. Two different eradication methods were selected. One room, in which most of the indirectly exposed sentinels had antibodies to MNV, was depopulated and thoroughly cleaned prior to repopulation. In the other room, in which only 13% of the sentinels had positive MNV titers, selective testing was used, and MNV-positive animals were removed. Data from surveillance of the sentinel mice exposed to dirty bedding indicate that the test-and-removal method was ineffective in eliminating MNV from the room, whereas sentinel mice in the room that underwent depopulation and cleaning prior to repopulation have not shown any evidence of MNV since December 2006.

Abbreviations: MNV, murine norovirus; PPE, personnel protective equipment

Murine norovirus (MNV), a nonenveloped single-stranded RNA virus, is a member of the *Caliciviridae* and is related to the human Norwalk-like viruses.[16] MNV was first described in 2003, when mice deficient in the Jak–STAT1 pathway had a high mortality rate and signs that are now known to correlate with MNV infection.[5] When mice deficient in the Jak–STAT1 innate immunity pathway were infected with MNV, they developed encephalitis, meningitis, cerebral vasculitis, focal interstitial pneumonia, peritonitis, pleuritis, and hepatitis.[5,8,14] Although the disease symptomatology of MNV differs markedly from the severe gastroenteritis seen in humans infected with Norwalk-like viruses, both the human and mouse diseases are predicted to spread by means of the fecal–oral route.[9,16] In addition, both diseases typically have acute onsets, cause mild enteritis, and are followed by rapid recovery in immunocompetent humans and animals, but persistent disease and shedding are possible, especially in immunocompromised patients.[3,8,9,11,14]

Currently, MNV is the most prevalent viral pathogen identified in laboratory animal facilities, and the full effect of the disease on research is not yet known.[4] According to studies to date and extrapolating from other viral infections, mice infected with MNV very likely have systemic alterations in their immune system. Infected mice, deficient in type I interferon receptors or components of the STAT1 pathway, accumulate MNV antigen in Kupffer cells in the liver and in macrophages and dendritic cells in the spleen.[5,14,15] MNV has been shown to cause splenic changes consistent with cellular stimulation and activation in immunocompetent mice.[8] In addition, when macrophages and dendritic cells are infected with MNV, they develop abnormal cell morphology, secrete IFNα, and may have alterations in translation activity.[15,16] The secretion of IFNα, an important cytokine for antiviral, antitumor, and immunomodulatory functions, may have a widespread effect, because IFNα regulates thousands of genes.[2,13] Many viruses alter the production of the interferons to escape the host immune system.[2,6,12,16] MNV alters the host cellular translational apparatus by binding the eukaryotic translation initiation factors eIF4G1 and eIF4E through the MNV protein VPg.[1] Studies also have shown that MNV persistently infects mice deficient in RAG2.[5,14] Given this mounting evidence of the effect of MNV on the immune system, the presence of MNV in a research facility likely will confound research results.

This case report describes the elimination of MNV from a barrier facility in which research with immunodeficient mice was being conducted. Only complete depopulation and disinfection prior to repopulation was successful in eradicating MNV. Limited testing and removal of positive animals was unsuccessful in permanently eradicating MNV.

Case Report

Enzootic MNV infection was identified in 2 of the 12 animal holding rooms in an AAALAC-accredited animal facility. This animal facility is run as a midlevel barrier with all mice either bred in the facility, purchased from the production facility of commercial rodent vendors, embryo-rederived into the facility, or shipped from a noncommercial contract facility. Each of the affected rooms housed approximately 800 cages of mice and served 40 to 50 scientific investigators. All animals were on protocols approved by the Animal Care and Use Committee in accordance with applicable federal regulations.

Animal husbandry. Mice were housed in individually ventilated (negative airflow, drawing air into the cage from the macroenvironment), sterile, microisolation caging (Thoren Caging Systems Hazleton, PA) with hardwood bedding (7086G, Harlan Teklad, Madison, WI) and nesting material (Nestlet, Ancare, Bellmore, NY). Mice were provided with an autoclaved pelleted rodent diet (2018SX, Harlan Teklad) and acidified water (pH 2.9). Clean cages were assembled with bedding, pelleted diet, and nesting material and then autoclaved to sterilize the contents. Acidified water, in a sterile water bottle, was added to the cage in the animal room prior to placing mice into the cage. Cages were changed weekly by using aseptic technique in a Class II biosafety cabinet. When the contents of the cages were

Received: 2 Aug 2007. Revision requested: 5 Sep 2007. Accepted: 23 Sep 2007.
National Institutes of Health, National Institute of Allergy and Infectious Diseases, Comparative Medicine Branch, Bethesda, MD.
Corresponding author. Email: rkastenmayer@niaid.nih.gov

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-02-25

handled, gloved hands and surfaces were saturated with 1:18:1 solution of a chlorine dioxide-based sterilant (Clidox, Pharmacal, Naugatuck, CT). Animal holding room temperatures were maintained between 20.0 and 23.3 °C, and relative humidity was maintained at 30% to 70%. The mice were housed on a 14:10-h light:dark cycle; the light intensity at cage level was less than 325 lx. All facility personnel wore dedicated shoes and scrubs and donned a surgical mask, hair bonnet, gloves, and disposable shoe covers. Personnel and scientific staff without dedicated facility clothing and shoes wore a high-density polyethylene (Tyvek, DuPont, Richmond, VA) jumpsuit and disposable shoe covers over their personal clothing, in addition to the surgical mask, hair bonnet, and gloves.

In addition to these precautions, access to all rooms was limited to the pertinent facility staff and scientists assigned housing in the room, to prevent spread of pathogens through the facility. Rooms in which sentinels had a serologic titer to MNV were placed on quarantine, and the quarantine procedures described were instituted.

Sentinel procedures. Outbred NIH Swiss Webster sentinels were exposed weekly for 18 wk to dirty bedding from cages containing experimental mice before being submitted for serology, parasitology, *Helicobacter* testing by fecal PCR, and necropsy, as previously described.[11] Each sentinel cage contained 3 mice, and a new mouse was placed in the sentinel cage every 6 wk at the same time the oldest sentinel was removed for testing. The viruses tested for were mouse hepatitis virus, Theiler murine encephalitis virus, mouse rotavirus, pneumonia virus of mice, Sendai virus, lymphocytic choriomeningitis virus, ectromelia virus, mouse cytomegalovirus, polyoma virus, reovirus 3, murine norovirus (MNV), mouse adenovirus, Hantaan virus, and murine parvoviruses. In addition, the following murine pathogenic bacteria were not found in the facility: cilia-associated respiratory bacillus, *Citrobacter rodentium*, *Clostridium piliforme*, *Corynebacterium kutscheri*, *Mycoplasma* spp., *Salmonella* spp., and *Streptobacillus moniliformis*. *Pasteurella pneumotropica* and *Pneumocystis murina,* although not included during routine testing, were never isolated from necropsied animals. With the exception of rare outbreaks of *Helicobacter* throughout the facility and of MNV in the 2 rooms described, all sentinels from this facility had been free from all mouse pathogens since the facility was first populated in 2002. Rooms with sentinels that were positive for excluded agents (that is, *Helicobacter* or MNV) were quarantined during the eradication efforts.

Testing. Serologic analysis was performed at the University of Missouri Research Animal Diagnostic Laboratory by using the microsphere-based serologic multiplexed fluorescent immunoassay as previously described.[4] RT-PCR–based testing was performed by using feces freshly collected from live mice or after euthanasia of mice, as previously described.[14] RNA was extracted from the supernatant of feces homogenized in sterile water by using Ultraspec-3 Isolation Reagent (Biotecx Laboratories, Houston, TX). Purified RNA was reverse-transcribed and amplified by using previously described primers[4] and the AccessQuick RT-PCR System (Promega, Madison, WI).

Quarantine housing and husbandry. All rooms that had a sentinel with a serologic titer to MNV were placed under quarantine during the period of confirmatory testing. Confirmatory testing consisted of evaluating the positive serum sample by indirect fluorescence assay.[4] In addition, cagemates of the MNV-positive sentinel were tested for the presence of MNV by serology and RT-PCR of feces or mesenteric lymph node. If these follow-up tests were negative, the room was returned to nonquarantine status. Rooms in which the follow-up testing confirmed the positive serologic result remained on quarantine.

Most of the animal shipments intended for quarantined rooms were redirected to other facilities or other areas of the same building; a few shipments of mice from commercial MNV-free facilities were allowed to enter the quarantined room for studies of short duration. The majority of the research procedures were performed by the facility staff using clean equipment. Any procedures performed by the investigative staff were reviewed by the facility management or veterinarian to ensure adherence to aseptic technique and the use of equipment and biologicals unexposed to MNV.

Increased security procedures for entering, handling cages, and processing items out of the room were instituted for the quarantined rooms. Personnel protective equipment (PPE) for quarantined rooms consisted of, in addition to the routine PPE, a high-density polyethylene jumpsuit with integrated booties over facility scrubs or the first jumpsuit, an additional pair of gloves and hair bonnet, and disposable shoe covers over the integrated booties. When handling cages on infected racks, personnel also donned high-density polyethylene sleeves. All paperwork leaving the room was sealed in clear plastic bags, which were sprayed with chlorine dioxide solution. The paperwork was copied, and the originals, still in the bag, were autoclaved and discarded. All equipment and used cages were rubber-banded shut, placed on a covered bulk truck, which was sprayed with chlorine dioxide solution prior to exiting the room, and immediately taken from the room to be autoclaved prior to routine cleaning. Any equipment that could not be autoclaved was saturated with chlorine dioxide solution for 10 min prior to removal from the room. On exiting the quarantined room, all personnel removed the additional PPE at the room threshold and immediately exited the facility. A shower and complete change of clothing were required to reenter the facility. Cage housing, temperature, humidity, and light cycle were unchanged in quarantined rooms.

Results

Eradication plan. The first step in evaluating the methods of eradication was to identify the source of the MNV infection. The strain and source of the cages on the quadrants that had a positive sentinel cage were reviewed, and the cage location was mapped. A common source for all positive cages in both of the 2 rooms was identified as a noncommercial contract facility that supplied mice to a limited number of investigators. This noncommercial facility subsequently was confirmed to be positive for MNV.[12] All shipments from this source were halted pending the eradication of MNV from its facility.

Depopulation of room A. In room A, 50 mice (20 breeding pairs and 10 animals used for experimental studies) had been received from the positive contract facility, and 13 of the 18 sentinels in the room (71%) had a positive MNV titer. Investigators in this room were not restricted to assigned racks, therefore cages containing the offspring of these 20 mated pairs were located randomly on all 6 racks in the room. Because the infection was widespread, testing of the mice on the affected racks was extremely expensive, and the effectiveness of test and removal eradication was uncertain, total depopulation was the only way to eliminate MNV rapidly from this room. The room was depopulated by transferring mice to other facilities in which MNV was not excluded. After full depopulation, all disposable items, HEPA filters, and prefilters were discarded, and the cage racks were disassembled. The rack motors and biological safety cabinet used for cage changing were wiped with chlorine dioxide solution and remained in the room. All equipment and rack parts

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-02-25

Table 1. Detection of MNV in breeder pairs and their offspring

	MNV serology results	Age (wk) at time of testing	Gender (no.) of mice/cage	Source
Breeder cage 1	Positive	30	Mixed (2)	External institution
Offspring cage 1-1	Positive	15	Female (3)	Breeder cage 1
Offspring cage 1-2	Negative	15	Male (2)	Breeder cage 1
Offspring cage 1-3	Positive	8	Female (2)	Breeder cage 1
Offspring cage 1-4	Negative	8	Male (1)	Breeder cage 1
Breeder cage 2	Positive	30	Mixed (2)	External institution
Offspring cage 2-1	Positive	15	Female (4)	Breeder cage 2
Offspring cage 2-2	Positive	15	Male (5)	Breeder cage 2
Offspring cage 2-3	Negative	11	Female (3)	Breeder cage 2
Breeder cage 3	Positive	30	Mixed (2)	External institution
Offspring cage 3-1	Negative	20	Female (4)	Breeder cage 3
Offspring cage 3-2	Negative	20	Male (3)	Breeder cage 3
Offspring cage 3-3	Negative	20	Male (3)	Breeder cage 3
Offspring cage 3-4	Positive	9	Female (4)	Breeder cage 3
Offspring cage 3-5	Positive	9	Male (3)	Breeder cage 3
Offspring cage 3-6	Negative	6	Female (4)	Breeder cage 3
Offspring cage 3-7	Negative	6	Male (3)	Breeder cage 3

were placed on a covered bulk truck and saturated with chlorine dioxide solution for 10 min. This bulk truck was allowed to sit in the room for 10 min before being taken immediately to the cage wash for sanitization in a tunnel or rack washer. The cage wash area was disinfected with chlorine dioxide solution once the equipment from room A was fully processed and before dirty cages or equipment from other parts of the facility were accepted. The walls and floors of the depopulated room were washed alternately with chlorine dioxide solution and rinsed with water 3 times. The racks were reassembled and recertified with new filters. The biological safety cabinet was fumigated with formaldehyde and recertified. Once these tasks were completed, the quarantine was lifted, and clean mice from approved sources were received into the room.

Identification and selective removal of positive animals in Room B. In room B, 3 of the 24 sentinels (13%) had a positive MNV titer, and 18 immunocompetent C57BL/6 mice expressing red fluorescent protein had been received from the positive contract facility. A portion of these mice were mated to form 3 breeder pairs that produced 44 offspring prior to identification of the positive MNV result in the sentinels. The offspring were housed in 14 cages on 3 quadrants of the 6 racks available in the room. Consultation with the researchers using the room led to the opinion that the infection was limited and showed no spread to the other racks in the room. Therefore, a method of identification and removal of infected cages and continued quarantine pending 6 mo of negative results in the sentinels was proposed to eradicate MNV from the room.

As part of the eradication of MNV from room B, all cages containing mice from the positive contract facility and their offspring were tested for MNV by serology using serum collected and pooled from all mice in a cage. All 3 breeder cages had positive MNV titers, as did 6 of the 14 offspring cages (Table 1). The presence of MNV-infected offspring indicated that the virus had been present in the breeder cages for at least 5 mo. The breeders in cage 1, which were presumed to be positive for MNV when they entered the facility at 3 wk of age, produced their first offspring when they were 15 wk old. These offspring and those born when the breeders were 22 wk old were serologically positive for MNV. In contrast, the 6-wk-old offspring from cage 3 were serologically negative; either the adults were no longer shedding virus, or the offspring had not yet seroconverted. Once all results from the serology and the RT-PCR assays were available, any mice that were descended from a breeder cage that was MNV-positive or that produced positive offspring were removed from the room, along with all mice in any sentinel cage to which they contributed bedding.

To ensure that MNV had not spread beyond these positive cages, all cages adjacent to MNV-positive cages were tested by serology or fecal RT-PCR, depending on the immune status of the mouse. None of the adjacent cages had evidence of MNV infection, suggesting that aseptic technique along with individually ventilated cages with filter tops had prevented the spread from adjacent cages during the cage change procedure.

However, 4 mo after the initial elimination of the known positive cages in room B, sentinel mice in 1 cage demonstrated a robust MNV titer. In addition, 2 cages of experimental mice that had contributed bedding to this sentinel cage were either seropositive or fecal RT-PCR–positive for MNV. Both cages previously had tested negative for MNV during the initial evaluation and removal of positive cages. These results suggested either an environmental nidus of infection or lack of detection during the first sampling round.

Efficacy of detection. During the testing process, 55 sentinel mice were serologically positive for MNV and underwent RT-PCR of mesenteric lymph nodes; 15 of the 55 mice (27%) were positive by both methods, suggesting either clearance of the virus from the lymph nodes, a viral load below the limit of detection of the RT-PCR assay, or mutations in the MNV that prevented detection by RT-PCR.

To determine the efficacy of detection, we concurrently tested cohoused sentinels that differed in age for the presence of MNV antibodies (Table 2). Several 12-wk-old mice that had been exposed to dirty bedding for only 6 wk were positive by multiplex fluorescent immunoassay, whereas their 24-wk-old cagemates with 18 wk of exposure were negative. Given data from the older sentinels only, 6 of the 13 known-positive cages would have been considered to be serologically negative for MNV.

Screening of animal shipments. To prevent the reintroduction of MNV, all sources of incoming mice were evaluated. Any mice

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-02-25

Table 2. Results of multiplex fluorescent immunoassay of cohoused sentinel mice of various ages and exposure periods

Cage	Age (exposure duration) of sentinel mice	
	24 wk (18 wk)	12 wk (6 wk)
1	Positive	Positive
2	Positive	Positive
3	Positive	Positive
4	Negative	Positive
5	Negative	Positive
6	Positive	Positive
7	Positive	Positive
8	Negative	Positive
9	Negative	Positive
10	Positive	Positive
11	Negative	Positive
12	Positive	Positive
13	Negative	Positive

shipped from a facility that had an MNV-positive result on the accompanying health report were denied entry. Mice shipped from commercial facilities for which MNV results were not reported were isolated and tested by antemortem serology or RT-PCR of the feces, as appropriate to their immune status. Further, 10% of the mice from each shipment were tested if the source room had not been tested within the previous month. These tests detected no positive animals. In addition, all sources of vendor-supplied animals reported negative MNV results for testing conducted in 2006 and 2007.

Discussion

This report is the first to describe the eradication of enzootic MNV from multiple rooms within a midlevel barrier animal facility. The virus initially was detected in sentinels exposed to dirty bedding, confirming the efficacy of this method for detecting MNV.[7] We used 2 different methods in the attempt to eradicate MNV from the 2 positive rooms within the facility. The method of total depopulation and decontamination of the room with chlorine dioxide solution was most expedient and minimized the risk of an unidentified source remaining in the room to perpetuate the infection. However, this method also required identification of alternative housing for hundreds of mice and euthanasia of nonessential mice. Chlorous acid, which is found in chlorine dioxide solution, has recently been shown to be effective in reducing the environmental concentration of MNV to less than 0.1%.[10] The alternative method of identification and removal of infected mice, although it initially appeared to be successful, was ultimately ineffective. This method required extensive and expensive testing as well as precise coordination between the veterinarian and technicians to identify positive cages and test all cages surrounding the positive cage. In addition, to ensure that an undetected nidus of infection did not remain within this room, 6 mo of quarantine was initiated after the first negative test result of all sentinels in the room. At 4 mo after the removal of all known-positive cages, 2 additional cages of experimental animals and 1 cage of sentinel mice seroconverted. This finding suggested that an environmental source of MNV likely was present, possibly associated with the racking system or the biological safety cabinet, and that a test-and-removal eradication system without environmental decontamination was ineffective in eliminating MNV.

This report demonstrates that MNV infection can persist in an active breeding cage of immunocompetent mice that are able to mount a robust antibody response. The breeder mice from room B produced MNV-positive offspring over a 20-wk period. Similarly, even if all positive cages are removed from the room, positive sentinel results could continue to occur unless the sentinel cage is eliminated and replaced with naïve animals. Future studies to determine the optimal sentinel detection procedures for MNV will be important to prevent missing hidden infections.

Because MNV has been recognized only relatively recently,[5] its full effect on research is undetermined. In our setting, the implications of endemic MNV infection presented an unwarranted research risk that needed to be eliminated. This report suggests that MNV, being persistent in certain strains and breeders, is not easily eradicated using a test and removal procedure. However, the virus can be eradicated by complete room depopulation and extensive decontamination.

Acknowledgments

We wish to thank Michelle Copeland and Leah Schmidt for coordinating the testing of hundreds of mice and the entire facility staff for their dedication and assistance during the quarantine period. We also are extremely grateful for the ongoing diagnostic testing support provided by the staff of the Division of Veterinary Resources at the National Institutes of Health, in particular Myrna Mandel, for providing the MNV RT-PCR results. This research was supported by the Intramural Research Program of the National Institutes of Health, National Institute for Allergy and Infectious Diseases. The views and opinions provided are those of the authors and do not reflect the official policy or positions of the National Institutes of Health, the Department of Health and Human Services, or the United States Government.

References

1. **Daughenbaugh KF, Wobus CE, Hardy ME.** 2006. VPg of murine norovirus binds translation initiation factors in infected cells. Virol J **3:**33–39.
2. **Der SD, Zhou A, Williams BRG, Silverman RH.** 1998. Identification of genes differentially regulated by interferon α, β, or γ using oligonucleotide arrays. Proc Natl Acad Sci USA **95:**15623–15628.
3. **Hsu CC, Riley LK, Wills HM, Livingston RS.** 2006. Persistent infection with and serologic cross-reactivity of three novel murine noroviruses. Comp Med **56:**247–251.
4. **Hsu CC, Wobus CE, Steffen EK, Riley LK, Livingston RS.** 2005. Development of a microsphere-based serologic multiplexed fluorescent immunoassay and a reverse transcriptase PCR assay to detect murine norovirus 1 infection in mice. Clin Diagn Lab Immunol **12:**1145–1151.
5. **Karst SM, Wobus CE, Lay M, Davidson J, Virgin HW.** 2003. STAT1-dependent innate immunity to a Norwalk-like virus. Science **299:**1575–1578.
6. **Katze MG, He Y, Gale M.** 2002. Viruses and interferon: a fight for supremacy. Nat Rev Immunol **2:**675–687.
7. **Manuel CA, Hsu CC, Riley LK, Livingston RS.** 2006. Soiled bedding sentinel detection of murine noroviruses [abstract]. Comp Med **56:**319.
8. **Mumphrey SM, Changotra H, Moore TN, Heimann-Nichols ER, Wobus CE, Reilly MJ, Moghadamfalahi M, Shukla D, Karst SM.** 2007. Murine norovirus 1 infection is associated with histopathological changes in immunocompetent hosts, but clinical disease is prevented by STAT1-dependent interferon response. J Virol **81:**3251–3263.
9. **Parashar UD, Quiroz ES, Mounts AW, Monroe SS, Fankhauser RL, Ando T, Noel JS, Bulens SN, Beard RS, Li J, Bresee JS, Glass RI.** 2001. "Norwalk-like viruses". Public health consequences and outbreak management. MMWR Recomm Rep **50(RR-9):**1–17.
10. **Park GW, Boston DM, Kase JA, Sampson MN, Sobsey MD.** 2007. Evaluation of liquid- and fog-based application of Sterilox hypochlorous acid solution for surface inactivation of human norovirus. Appl Environ Microbiol **73:**4463–4468.

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-02-25

11. **Perdue KA, Green KY, Copeland M, Barron E, Mandel M, Sosnovtsev SV, Elkins WR, Ward JM.** 2007. Naturally occurring murine norovirus infection in a large research institution. J Am Assoc Lab Anim Sci **46:**39–45.
12. **Prévôt D, Darlix J, Ohlmann T.** 2003. Conducting the initiation of protein synthesis: the role of eIF4G. Biol Cell **95:**141–156.
13. **Takaoka A, Yanai H.** 2006. Interferon signaling network in innate defense. Cell Microbiol **8:**907–922.
14. **Ward JM, Wobus CE, Thackray LB, Erexson CR, Faucette LJ, Belliot G, Barron EL, Sosnovtsev SV, Green KY.** 2006. Pathology of immunodeficient mice with naturally occurring murine norovirus infection. Toxicol Pathol **34:**708–715.
15. **Wobus CE, Karst SM, Thackray LB, Chang K, Sosnovtsev SV, Belliot G, Krug A, Mackenzie JM, Green KY, Virgin HW.** 2004. Replication of norovirus in cell culture reveals a tropism for dendritic cells and macrophages. PLoS Biol **2:**e432.
16. **Wobus CE, Thackray LB, Virgin HW.** 2006. Murine norovirus: a model system to study norovirus biology and pathogenesis. J Virol **80:**5104–5112.

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-02-25