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Naturally Occurring Murine Norovirus Infection in a Large Research Institution

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Murine norovirus (MNV) is a recently discovered infectious agent in mice and may be the most common naturally occurring infection of laboratory mice in North America. In 2005, we surveyed the Swiss Webster female sentinel mice in our institute's research facilities. Of the 4 facilities surveyed, 3 had sentinel mice that were positive for MNV antibodies, whereas our largest facility (which only receives mice directly from select vendors or by embryo rederivation directly into the facility) was apparently MNV-free. However, testing of sentinel mice in this large facility 1 y later found that 2% of the animals had developed MNV-specific antibodies. In a recently opened fifth facility, a serologic survey in 2006 identified MNV-antibody–positive Tac:SW sentinel mice that had received bedding from experimental mice on the same rack quadrant. Reverse transcription–polymerase chain reaction analysis of feces from the cages of these mice showed evidence for shedding of MNV. These sentinel mice were used to study the fecal excretion, antibody development, gross lesions upon necropsy, histopathology, and immunohistochemistry of the viral infection. None of the MNV-antibody–positive sentinel mice exhibited clinical signs or gross lesions, but these mice excreted virus in feces and developed antibodies to MNV. Histopathologic lesions consisted only of a few hepatic inflammatory foci in each liver section, some of which were immunoreactive with antibodies to MNV. MNV viral antigens also were present in the mesenteric lymph nodes.

Abbreviations: MNV, murine norovirus; IHC, immunohistochemistry; NIAID, National Institute of Allergy and Infectious Diseases; RT-PCR, reverse transcription–polymerase chain reaction; NCCS, noncommercial contract source

Noroviruses are nonenveloped single-stranded RNA viruses in the family Caliciviridae. Infection of mice with norovirus was first recognized and described in a colony of RAG2/STAT1$^{-/-}$ mice.[7] Lethality of the new norovirus, designated murine norovirus (MNV), in these mice was linked to the absence of the *Stat1* gene, because mice lacking only the *Rag2* gene became chronically infected but did not die.[7] In addition, the researchers showed that the combined absence of the interferon αβ and γ receptor genes resulted in high mouse mortality. Subsequent studies reported clinical signs and histologic lesions in several additional genotypes of immunodeficient mice.[16] MNV also has the potential to infect rodent biologicals, because the virus can grow in cultured macrophages and dendritic cells.[17] Other than causing decreased fecal-content weights in 129S6/SvEvTac mice,[10] the virus has been clinically silent in the immunocompetent mice studied thus far,[4,5,7,8] although some MNV strains persist in immunocompetent mice for at least 8 wk after experimental infection.[4]

Because of the ability of MNV to confound in vivo immunology research and infect cell cultures, the extent of infection in our colonies and the effectiveness of our dirty bedding sentinel program in detecting the virus were of interest. We evaluated the fecal excretion, antibody development, necropsy, histopathology, and immunohistochemical findings of female Tac:SW sentinel mice naturally infected with MNV after weekly exposure to dirty bedding. Potential methods for eliminating the virus and the probability of maintaining noninfected colonies free of MNV in a research setting were explored as the project progressed.

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Materials and Methods

Sentinel mice. Outbred, 4- to 6-wk-old, female Swiss Webster (Tac:SW) mice (Taconic, Germantown, NY) were used as sentinels. Sentinel animals were ordered in lots of 30 to 225 animals that shipped from the vendor in crates containing 25 mice per container. These sentinels were housed in 5 facilities, designated Facility 1 through 5, in the same manner as the mice they monitored. All facilities were accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International.

The caging type and bedding used varied slightly among the different facilities. Although the majority of our mouse colonies are housed in individually ventilated cages (Thoren Caging Systems, Hazleton, PA; Alternative Design Manufacturing and Supply, Siloam Springs, AR; Lab Products, Seaford, DE), static Micro-Isolators™ (Lab Products) are used in 2 facilities. Sentinels were fed rodent chow (NIH31 diet, Zeigler Brothers, Gardners, PA) ad libitum. Bedding typically was hardwood chips (Nepco, Warrensburg, NY), but corncob bedding (Harlan Teklad, Madison, WI) was used in some rooms of 1 facility. All sentinel mice were provided with nesting material (Nestlet, Ancare, Bellmore, NY) and ad libitum acidified water (pH, 2.9 ± 0.2). Room temperatures were maintained between 18.9 and 24.4 °C, and relative humidity was maintained between 30% to 70% with rare deviations. The mice were housed on a 14:10-h light:dark cycle with a light intensity at cage level of less than 325 lux. Sentinel mice were on a protocol approved by the institutional animal care and use committee in accordance with applicable federal regulations.

In all 5 facilities, each mouse rack contains 4 sentinel cages (1

Table 1. Characteristics of mouse colonies evaluated

Facility	Caging	Average daily cage inventory	Breeding cages (%)[a]	Health and animal receipt[b] history	March 2005[c]		April 2006[c]	
					No. MNV-positive/ total no.	% positive	No. MNV-positive/ total no.	% positive
1	IVC	729	2	Older facility with 1 mouse room that was depopulated and repopulated in February 2004; accepts vendor animals, occasional transfers, and rare imports.	5/24	21	7/24	29
2	IVC except 1 room with SMI	6886	11	Older facility in operation for more than 20 y, with rooms depopulated occasionally; accepts vendor animals, transfers from other NIH facilities, and occasional imports.	174/256	68	175/260	67
3	IVC	7479	12	Facility opened in early 2002; accepts vendor animals and animals from specialty contracts; others are embryo-rederived in.	0/197	0	5/225	2
4	SMI	2539	9	Older facility that has been in operation for 20+ years with racks depopulated rarely; accepts vendor animals, occasional transfers and imports.	85/105	81	85/103	83
					March 2006[c]		July 2006[c]	
5	IVC	1668	8	New facility opened in mid-October 2004 with several breeding colonies from Facility 2 and animals from Facility 4. A large shipment of imported mice was received shortly after opening. Accepts vendor animals and commonly receives transfers from our and other facilities for rederivation.	28/69	41	33/67	49

IVC, individually ventilated cages; SMI, static microisolation caging.
[a]Percentage of breeding cages was based on the number of breeding cages held to the total number of cages in the facility.
[b]See Table 2 for details on the impact of animal source on the inhouse mouse population.
[c]Positive serology findings are shown both as the number of serologically positive sentinel mice to the total number of sentinel mice, and as a percentage of positives.

for each quadrant of the rack), each housing 3 mice. This setup results in a maximum of 34 cages of research mice providing used bedding to 1 sentinel cage. Each week, 15 cm^3 of used bedding is collected from each experimental mouse cage within a quadrant and placed into a cage that will house the sentinels for that quadrant. Animals are observed daily for abnormalities. All sentinels that exhibit clinical abnormalities, except for certain traumatic injuries, are expeditiously submitted for a diagnostic workup. Health surveillance testing is performed every 6 to 7 wk on the oldest mouse in the cage, which is submitted live to the diagnostic laboratory. A new 4- to 6-wk-old sentinel animal is added to the sentinel cage whenever one is removed. Sentinel animals were 22 to 27 wk old and had received dirty bedding for 18 to 21 wk by the time they were sent to the reference laboratory (Division of Veterinary Resources, National Institutes of Health, Bethesda, MD) for a health surveillance workup. Sera from the mice were sent to the Research Animal Diagnostic Laboratory at the University of Missouri (Columbia, MO), whereas feces and tissues were sent to inhouse laboratories.

During the study period, the sentinel mice in all facilities were devoid of antibodies to a panel of mouse viruses (mouse hepatitis virus, pneumonia virus of mice, Sendai virus, Theiler murine encephalomyelitis virus, mouse rotavirus, lymphocytic choriomeningitis virus, ectromelia virus, mouse cytomegalovirus, minute virus of mice, polyoma virus, reovirus 3, mouse adenovirus, Hantaan virus, and mouse parvovirus), cilia-associated respiratory bacillus, *Citrobacter rodentium*, *Clostridium piliforme*, *Corynebacterium kutscheri*, *Salmonella* sp., *Streptobacillus moniliformis*, *Mycoplasma pulmonis*, and common endo- and ectoparasites. *Helicobacter* sp. is present in all facilities except Facility 3. The sentinel mice were not tested for MNV infection prior to placement.

Research mouse colonies. Colonies were housed in the caging described above. Facility 4 uses only static microisolation caging in the surveyed rooms, and Facility 2 contains 1 room with an approximate average daily inventory of 550 static cages. Information regarding average daily mouse cage inventory, percentage of breeding cages, and mouse source for each facility was determined through analysis of data captured by our custom electronic inventory system. Rack, room, and facility depopulations during the last 10 y were considered. Depopulation information was available from archived sentinel test results and quarterly reports composed and maintained by our animal quality assurance unit. This information, along with a short description of the historical health status and source of the animals held, is provided for each facility in Tables 1 and 2.

Serology. Antibodies to MNV were detected by a microsphere-based serologic multiplex fluorescent immunoassay (Research Animal Diagnostic Laboratory) that cross-reacts with 4 known murine norovirus strains.[4,5] In March 2005 and April 2006, a combined total of 1194 Tac:SW sentinel mice in 4 facilities (designated Facilities 1 through 4) were serologically surveyed. Whereas most of the sera collected in April 2006 were tested for MNV antibodies shortly after collection, the sera from mice in Facility 3 were frozen and stored at –80 °C for approximately 22 wk before being processed. During March and July 2006, 69 and

Table 2. Sources of the typical inhouse mouse population by facility

	Facility 1		Facility 2		Facility 3		Facility 4		Facility 5	
	No. of cages	% of total	No. of cages	% of total	No. of cages	% of total	No. of cages	% of total	No. of cages	% of total
Vendors	335	56.6	1880	27.1	2494	32.4	406	15.9	345	19.8
Foreign and domestic imports	0	0.0	30	0.4	0	0.0	24	0.9	58	3.3
Transfers from NIH sources	152	25.7	240	3.5	0	0.0	51	2.0	429	24.6
Inhouse breeding[a]	105	17.7	4796	69.0	5212	67.6	2078	81.2	914	52.3
		100%		100%		100%		100%		100%

NIH, National Institutes of Health.

[a]Mice from all other sources contribute to the percentage of inhouse breeding. Animals rederived into Facility 3 are counted as being bred inhouse.

67 sentinel mice, respectively, from Facility 5 were serologically surveyed for antibodies to MNV.

Reverse transcription–polymerase chain reaction (RT-PCR) and sequence analysis. Feces were collected in April and August of 2006 for analysis of viral shedding from sentinels in Facility 5. In April 2006, feces were obtained from 28 sentinel cages. The cages chosen were those containing sentinels that had MNV antibodies when surveyed in March 2006. In August 2006, fecal samples were collected from 50 sentinel cages that were housed in the 3 rooms that had MNV-positive sentinel serology results when surveyed the previous July. Additional fecal samples were selected randomly from MNV-positive sentinel mice housed in 4 of the facilities. These samples were used for RNA extraction and sequence analysis.

For these analyses, a 10% suspension in water was made from each fecal sample, vortexed, and centrifuged twice. From a 100-µl aliquot of each supernatant, RNA was extracted by use of guanidine salts, urea, detergent, and phenol (Ultraspec 3 Isolation Reagent, Biotecx Laboratories, Houston, TX). The purified RNA was amplified by RT-PCR using the AccessQuick RT-PCR System (Promega, Madison, WI) and a primer pair designed to generate a MNV1 polymerase gene (Genbank accession #AY228235) product corresponding to nucleotides 4228 through 4545.[5] Toward the end of the project, we substituted a more inclusive pair that amplified a product from nucleotides 5473 through 5659 and could detect the recently identified MNV2, 3, and 4 in addition to MNV1.[4]

The RT-PCR products were electrophoretically separated on a 3.25% NuSieve 3:1 agarose (Cambrex Bio Science, Rockland, ME) gel stained with ethidium bromide. A subset of representative MNV strains from 4 facilities (1, 2, 3, and 5) were analyzed further by sequencing of a region of the RNA genome corresponding to nucleotides 4556 through 4788 as described previously.[16] Briefly, RT-PCR was used to generate a DNA product from the viral RNA extracted from feces or tissues. The DNA product was gel-purified and sequenced directly. The sequences were compared with the corresponding regions of strains MNV1 (Genbank accession number, AY228235), MNV2 (DQ223041), MNV3 (DQ223042), MNV4 (DQ223043), and Berlin (DQ911368). Multiple alignments were performed by using the Clustal W program (MacVector, Accelrys, San Diego, CA), and phylogenetic analysis was carried out using the neighbor-joining method[11] with distances estimated by the Jukes-Cantor parameter method.[6]

Pathology. A complete necropsy was performed on the 67 Tac:SW sentinel mice from Facility 5 that were surveyed in July 2006. Liver, spleen, mesenteric lymph node, and small intestine were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Immunohistochemistry (IHC). Sections of liver, spleen, small intestine, and mesenteric lymph node from 33 Tac:SW mice from Facility 5 that were serologically positive for MNV during the July 2006 survey were examined by IHC. The primary MNV antiviral antibodies used in the IHC included those raised against recombinant MNV1 capsid protein in guinea pigs (serum diluted 1:2000) or recombinant MNV1 proteinase–polymerase protein in guinea pigs (serum diluted 1:500).[12] Antigen retrieval from fixed tissues was performed in a food steamer (Sunbeam, Boca Raton, FL) with Diva Decloaker Solution (Biocare, Concord, CA). For visualization of signal, a commercial kit (Vector Guinea Pig ABC kit, Vector Labs, Burlingame, CA) was used; diaminobenzidine (DAB) was the chromogen with hematoxylin as the counterstain. The chromogen Bajoran Purple (Biocare) was used also, for comparison.

Because Facility 5 does not exclude MNV and because replacement sentinels were housed in a room containing MNV-positive animals, known uninfected age-matched control mice from Facility 5 were not available for IHC. Although replacement sentinels might have been negative, we could not prove non-infection without extensive studies. Instead, appropriate MNV viral antigen-positive and -negative control slides from livers of noninfected and MNV experimentally infected mice were included, as done previously,[16] as well as livers from control mice for an experimentally induced MNV infection study.[6,16] These known uninfected livers did not show Kupffer cell MNV antigen expression.

Results

Research colony health and animal receipt history. Facility 1 contains only 1 mouse holding room, which was completely depopulated and repopulated in 2004 after an outbreak of parvovirus. Facility 2 contains 11 mouse-holding rooms, of which 1 room was completely depopulated in 2003. Facility 3 had no room depopulations before the detection of MNV in 2 of 10 rooms. Facility 4 has 8 rooms, none of which had been completely depopulated during the 10-y period although partial racks were depopulated in 1999 and in 2000 after pinworm and mouse adenovirus outbreaks, respectively. Facility 5 has not had any colony depopulations since its opening in October 2004.

Animal source information is summarized in Table 2. The source data for mice held in Facility 3 are slightly skewed as a result of depopulating the MNV-serology-positive mice from that facility.

Prevalence of MNV antibodies in sentinel mice. Data from the 1194 Tac:SW sentinel mice housed in Facilities 1 through 4 and tested in 2005 and 2006 and from the 69 and 67 Tac:SW sentinel mice that were sampled for MNV infection in the newly opened Facility 5 during March and July 2006, respectively, are summarized in Table 1. The number of MNV-positive sentinel animals per facility ranged from 2% (Facility 3) to 83% (Facility 4) of those tested in a given year.

Pathology of tissue samples from Tac:SW sentinel mice. Tissues from all 67 mice in Facility 5 that were necropsied appeared

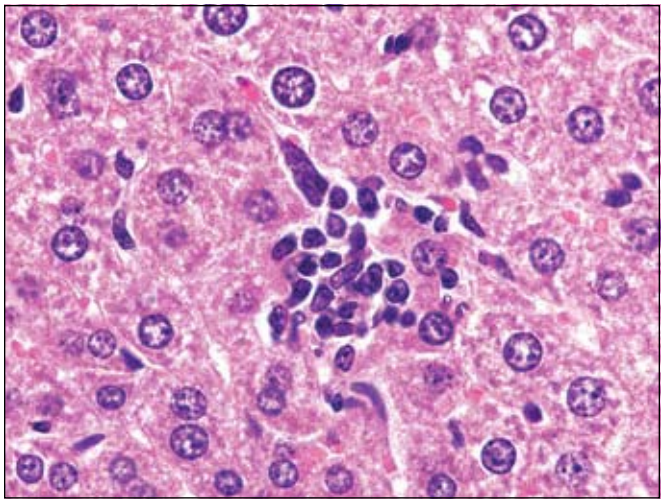

Figure 1. Liver of an MNV antibody-positive Swiss Webster sentinel mouse with a focus of inflammatory cells, including lymphocytes and a few macrophages. Hematoxylin and eosin stain; magnification, ×1000.

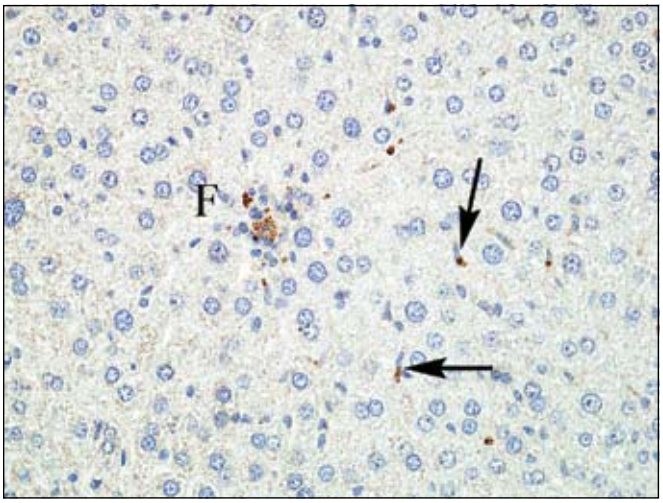

Figure 2. Liver with a small focus (F) of inflammatory cells showing MNV capsid antigen in the focus and in some Kupffer cells (arrows). Immunohistochemistry, hematoxylin stain; magnification, ×400.

grossly normal. Histologically, the mesenteric nodes were normal, although some nodes had more germinal centers than others. The livers appeared mostly normal histologically, but 80% of the livers showed a few small foci of inflammatory cells including lymphocytes, macrophages, or neutrophils in each liver section (Figure 1). Although foci of hypertrophic Kupffer cells were present, lesions were not numerous enough for a diagnosis of hepatitis. Lesions were not observed in the spleen or small intestine.

Detection of MNV-antigen–positive cells in sentinel mice by IHC. Of the 67 sentinel mice from Facility 5 that were necropsied, 33 MNV-antibody-positive mice were analyzed further by IHC. Antibodies specific for the MNV1 capsid or proteinase–polymerase antigens immunoreacted with Kupffer cell or small inflammatory cell foci (or both) in the livers of 30 mice and in the paracortex of the mesenteric lymph nodes of 11 mice (Figures 2 to 6). The numbers of immunoreactive hepatic Kupffer and inflammatory cells varied among cases. Of the 33 MNV serologically positive mice, we found that 25 had a total of 73 small hepatic inflammatory foci composed of small lymphocytes, neutrophils, or macrophages. Of these 25 mice, 9 had MNV-positive inflammatory cells in the foci. All 25 mice with inflammatory cell foci in the liver had immunoreactive Kupffer cells focally, multifocally, or diffusely. Usually only a few cells in each focus were immunoreactive, but in some cases many Kupffer cells were focally positive (Figure 2). Some hypertrophic Kupffer cells expressed viral antigens, but most Kupffer cells were morphologically normal in sections stained with hematoxylin and eosin. To differentiate the brown pigment occasionally found in Kupffer cells from the brown immunoreactive staining of an antigen–antibody reaction (Figure 3, with diaminobenzidine as the chromogen), we subsequently used the chromogen Bajoran Purple (Figure 4). In liver sections of uninfected mice, no immunoreactivity was seen in the liver.[16] Control slides of samples from infected sentinel mice to which no primary antibody was added also did not immunoreact.[16] In the sections of mesenteric lymph nodes (Figures 5 and 6), usually 1 to 5 cells in the paracortex were immunoreactive. A few nodes had immunoreactive cells within germinal center cells (Figure 6). MNV antigens were seen in dendritic-like cells, macrophages, and other unidentifiable cells. No such immunoreactivity was seen in livers in the absence of the primary MNV antibodies.

Identification of MNV in sentinel mice by RT-PCR and sequence analysis. In the April 2006 survey (of Facility 5), 8 (28.5%) of the 28 fecal samples taken from cages with positive MNV serology results were MNV-positive by RT-PCR (data not shown). In the August 2006 collection, 4 (8%) of the 50 fecal samples taken from the 3 rooms with positive MNV serology results in July were MNV-positive by RT-PCR. Selected samples in 4 of the 5 facilities were examined further by sequence analysis of RT-PCR products amplified from either feces or tissue extracts. The viruses in each facility were distinguishable as unique strains, although the overall nucleotide identities were high (ranging from 90% to 98% identity). Phylogenetic analysis of the nucleotide sequences in comparison with previously characterized MNV strains confirmed that genetic variation occurred among the strains within our institute (Figure 7). However, this analysis also showed that the viruses detected in Facilities 1 and 2 were closely related (ranging from 96% to 98% identity. Of interest, the virus in Facility 5 was highly related (99% identity) to a strain detected in the feces of mice from a noncommercial contract source (NCCS).

Discussion

Health surveillance of large research colonies housed in individual caging units is difficult and expensive. Although not ideal for detecting some adventitious rodent agents known to confound research results,[1-3,13] serology testing of sentinel mice housed on dirty bedding from cages of research mice is the most common method of monitoring rodent colonies at the National Institutes of Health. All of the facilities operated by the National Institute of Allergy and Infectious Diseases (NIAID; National Institutes of Health, Bethesda, MD) primarily hold genetically manipulated mouse strains, with a large percentage of the mice having a modified immune system. Owing to the effect of MNV on animals with innate immunity deficits and the nature of the research performed at our institution, it was desirable to know the extent of MNV infection in our colonies and the effectiveness of our dirty-bedding sentinel program to identify infection. An effective program enabled us to study viral prevalence and spread in our colonies with the objective of identifying and instituting biosecurity procedures to ensure the biostability of our research animals.

Swiss Webster mice are commonly used as sentinels throughout the National Institutes of Health intramural program in research settings where the number of genetically manipulated and immune deficient mouse strains makes the use of same-strain sentinels impractical. An outbred all-purpose stock, Swiss Web-

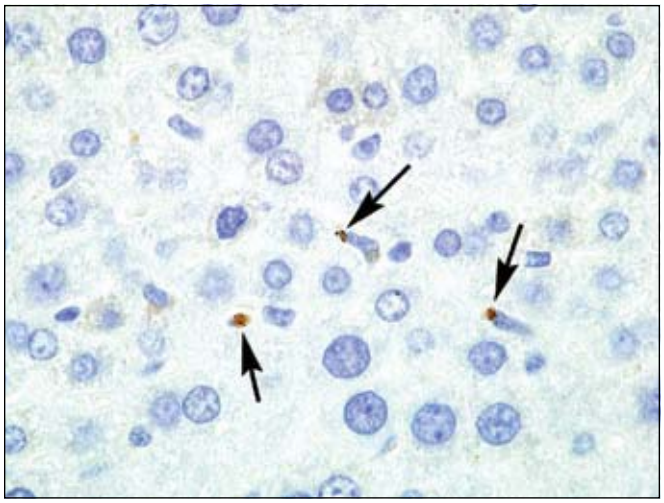

Figure 3. MNV capsid antigen in Kupffer cells (arrows) in liver of sentinel mouse. Immunohistochemistry; hematoxylin stain, with diaminobenzidine as the brown chromagen; magnification, ×1000.

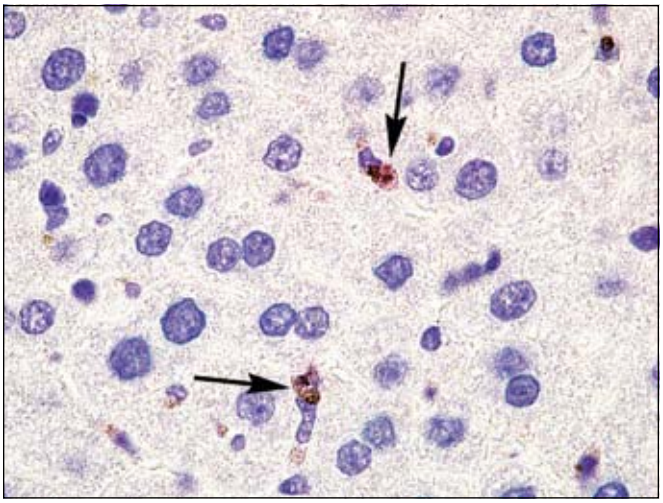

Figure 4. MNV protease–polymerase antigen in 2 Kupffer cells showing purplish color (arrows) to distinguish the color from natural pigment. Immunohistochemistry; Bajoran Purple as the purple chromagen; magnification, ×1000.

ster mice have the ability to mount a robust antibody response to adventitious infectious agents of concern. We also note minimal fighting among the mice with cage population turnover.

MNV was first recognized in 2003, but a commercial serology test was not available until 2005;[5] the first year that the vendor tested their colonies was 2006. The Tac:SW mice we received during the time period of this report were not tested for MNV either at the vendor facility or prior to placement as sentinels. Although some of the mice may have been infected with MNV prior to exposure to dirty bedding, the pattern of MNV positivity in our facilities makes prior infection highly unlikely. All of the sentinel mice came from the same vendor, where multiple litters were pooled together to fill orders. Replacement animals all arrive on the same day, in containers holding 25 mice each. Over the course of a single survey rotation, facilities at our institution receive 620 to 700 mice for sentinel use within a 2.5-wk period; Facility 3 alone receives approximately 225 replacement mice that all arrive on the same day. Approximately 10% of the age-matched animals received are held as replacements in the event an active sentinel becomes sick or dies. These replacement sentinels are treated as research mice during cage changing; that is, dirty bedding from their cages is added to the sentinel cage with each cage change. Although possible, the likelihood that shipments of Tac:SW mice to Facility 3 before the March 2005 testing were all MNV-negative while those for the other facilities were not is highly unlikely. Conversely, the odds of any single facility consistently receiving MNV-negative mice from a source while the others receive positive ones are extremely small. Of the more than 200 active sentinels in Facility 3 when the 2% MNV prevalence was identified, 13 of the 22 sentinels in 1 room were MNV-positive, and all positive cases were linked to cages of mice from the NCCS. In a second room, 3 of the 23 sentinels were MNV-positive; again, in all cases, mice from the NCCS were housed in the rack quadrants.

Although other authors have used serologic and RT-PCR methodologies to demonstrate infection of immunocompetent mice,[4,5,7,9,10] the present study is the first to report large-scale serologic, histologic, and IHC findings in immunocompetent Swiss Webster mice infected with field strains of MNV through exposure to dirty bedding. Infection was characterized by a robust antibody response, and neither consistent gross nor histopathologic changes were present in the tissues of the mice studied. Sentinel mice commonly had small hepatic inflammatory foci, some of which had MNV viral antigens in the inflammatory cells, but many did not. Inflammatory foci often are found in the livers of mice at all ages. However, according to our findings in infected sentinels and infected immunodeficient mice, some of these foci may be due to MNV infection.[16] Other possible causes of these foci include *Helicobacter* infections and other unknown etiologies. RT-PCR identified viral RNA in feces, and IHC revealed viral RNA in the mesenteric nodes and liver of serologically positive mice.

We used serology to monitor the extent of MNV infection in our facilities over time. Viral prevalence as determined by serology was relatively stable in Facilities 1, 2, 4, and 5 during the time studied; there was no depopulation of rooms during this period. No effort was made to eliminate MNV from positive racks identified in March 2005. In most cases, the same rack quadrants that were serologically positive in 2005 still tested positive in April 2006; the same was true for sentinel cages that were negative. Discrepancies in positive and negative sentinel results were attributed to movement of cages on a rack side.

Wild-type 129 mice inoculated orally with CsCl-purified MNV1 inoculum developed a detectible antibody response 14 to 21 d postinfection.[7] In addition, 20% of Hsd:ICR (CD1) mice given purified MNV1 orally were seropositive at 1 wk and 70% were seropositive by 2 wk.[5] MNV is stable in feces held at room temperature for 2 wk,[9] and viral persistence for as long as 8 wk has been demonstrated in Hsd:ICR(CD1) mice.[4] The exact time of MNV exposure and infection in our sentinels is unknown, but they likely were infected within 1 to 2 wk of being placed into a positive sentinel cage, either by exposure to infected animals already in the sentinel cage or from contaminated bedding being introduced into the cage weekly. Fecal shedding of MNV occurred in 2 of 15 and 10 of 15 Tac:SW mice housed for 5.3 wk and 5.6 wk, respectively, in sentinel cages that consistently tested MNV-positive (data from Facility 3, not shown). These findings are similar to those of Manuel and colleagues,[9] in which all 4- and 8-wk-old ICR mice in a simulated soiled-bedding sentinel study were fecal RT-PCR–positive 4 wk postexposure. For these same 2 groups of MNV-fecal–positive sentinels at our institution, 13 of 23 and 15 of 22 were MNV-seropositive; all of the mice that were fecal RT-PCR–positive were also seropositive.

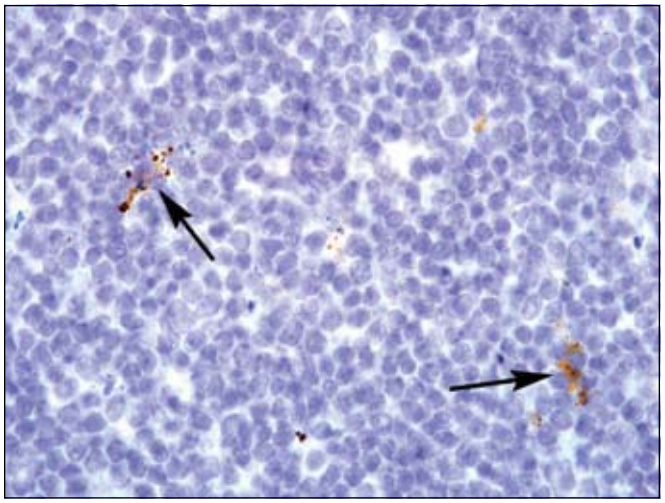

Figure 5. Mesenteric lymph node showing MNV capsid antigen in 2 foci (arrows), probably 2 dendritic cells. Immunohistochemistry; hematoxylin stain; magnification, ×1000.

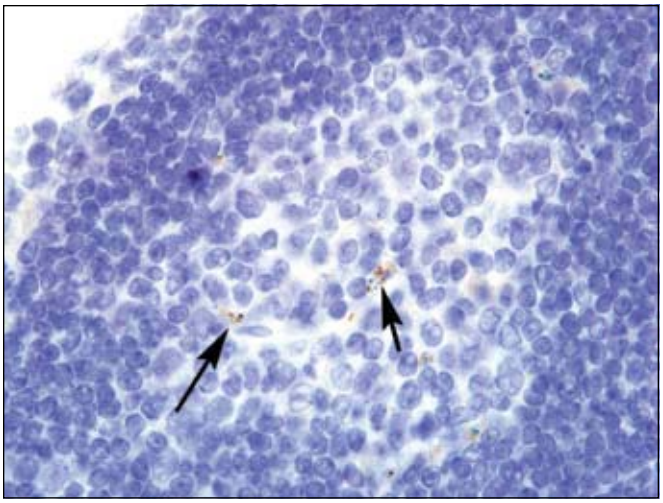

Figure 6. Mesenteric lymph node with MNV capsid antigen in germinal center cells (arrows). Immunohistochemistry; hematoxylin stain; magnification, ×1000.

New sentinels, with rare exception (for example, those with clinical problems or that die prematurely), are scheduled for health surveillance on the third (6- to 7-wk) rotation of the program. The 11 mice necropsied in July 2006 and shown by IHC to have MNV were housed for 18 wk in cages that were MNV-serology–positive when tested in March 2005. In light of the observation noted in Facility 3—that new sentinels placed into positive cages shed MNV 5 to 6 wk after placement—the IHC-positive finding at 18 wk postplacement suggests that the mice were reinfected with the same or a different strain of MNV or, as postulated by Hsu and colleagues,[4] immunocompetent mice remain persistently infected with MNV longer than 8 wk.

The sera collected in April 2006 from Facility 3 were not screened for MNV antibody until September 2006. Another MNV serology test of all sentinels in Facility 3 was performed in mid-September 2006 (data not shown); all positive findings were in the 2 rooms previously found to be positive. The ability to contain the virus was exemplified by confinement of MNV in the original 2 positive rooms for longer than 22 wk despite the housing of replacement sentinels, for use throughout the facility, in 1 of the affected rooms.

We used animal receipt logs to determine a common source of the mouse strains housed on the positive sentinel quadrants in Facility 3. We traced the 2% infection rate to a NCCS; these animals were the only ones received that did not come from a vendor or were not embryo-rederived into the facility. All positive sentinel mice found in Facility 3 surveyed rack quadrants that, at some time, had housed mice from the NCCS. We contacted the NCCS, who submitted serum and feces to the same diagnostic laboratories that we used. The samples submitted tested positive for MNV.

MNV1 capsid or protease–polymerase antigens were present in the mesenteric lymph nodes or livers (or both) of Tac:SW sentinel mice from Facility 5 that were surveyed in July 2006. Although Facility 5 was not tested for MNV in 2005, the facility originally was populated with animals that had been moved from positive rooms in Facility 2, a large breeding colony of mice that has been in existence since the 1960s and that since has been shown to harbor MNV, and transgenic mice that originated from the NCCS. Considering the high prevalence rate of MNV in the source colonies it is reasonable to assume that Facility 5 was contaminated with MNV shortly after opening.

Sequence analysis of representative strains from each facility (with the exception of Facility 4) showed evidence of genetic variation among the strains in our institute. However, the viruses detected in Facilities 1 and 2 were highly related, suggesting a possible common source. In addition, the virus in Facility 5 was nearly identical to that of a virus detected in the NCCS. Genetic typing of MNV strains in animal facilities likely will prove useful in tracking the sources of MNV viruses into MNV-free animal rooms, and give insight into the spread of viruses in a colony.

Although MNV might be expected to spread rapidly through conventionally housed mouse colonies, viral spread—either within rack quadrants, between racks in the involved rooms, or between rooms—did not occur in Facility 3 from April 2006, when the first 2% sentinel seroconversion occurred, and September 2006, when the sentinels were retested serologically (data not shown). Use of microisolation caging, strict microisolation cage-changing technique performed in a biological safety cabinet, the requirement to perform technical procedures in a safety cabinet, use of disposable clothing donned when entering and removed on exiting a room, and prohibition of mouse movement from a laboratory back into the animal facility likely all contributed to the prevention of viral spread.

The sentinel, animal population, and facility data compiled in Table 1 allowed us to infer information about the spread of naturally occurring MNV in facilities at our institution and to deduce effective methods for excluding the virus. The percentage of serologically positive mice was lowest in Facility 3. The lack of MNV infection in Facility 3, other than the outbreak for which the putative source was identified, supports the claim that embryo rederivation is a successful method for exclusion of MNV.[14] Lack of seropositive sentinel mice in rooms of Facility 3 that have accepted animals directly from 4 commercial vendors since 2002 also adds support to claims that their colonies are free of MNV.

The interplay of factors such as facility time in operation, existence of permanent breeding colonies, acceptance of mice from noncommercial and known-positive sources, and room depopulation efforts for other outbreaks can be expected to affect the prevalence of MNV. Increased prevalence of MNV occurred in those of our facilities that had been in operation for at least 20 y, held long-standing breeding colonies (some were established prior to 1989, when mice were held in open caging and changed on a tabletop), and accepted mouse transfers and domestic and foreign imports.

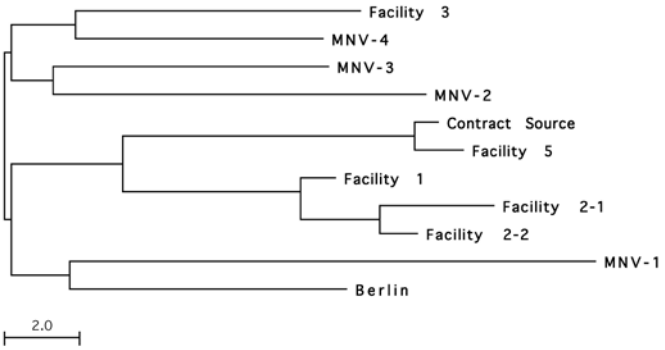

Figure 7. Phylogenetic analysis of the relationship of selected MNV strains from 4 facilities in this study. The strains are designated according to the facility of origin; 2 viruses (designated 2-1 and 2-2) were analyzed from Facility 2. A region of the genome corresponding to nucleotides 4556 through 4788 in the RNA-dependent RNA polymerase coding sequence was used in the analysis. Multiple sequence alignment of the corresponding sequences and phylogenetic tree reconstruction were performed using ClustalW and neighbor-joining methods, respectively. The phylogenetic tree has been midpoint-rooted, and the branch lengths are drawn to scale. The scale bar indicates the distance corresponding to 2 nucleotide changes.

In light of the immunology research being performed, the low prevalence of MNV in Facility 3, the ability to trace the source of the 2% infection rate through sentinel data and animal receipt logs, the lack of viral spread between ventilated cages over a 22-wk period, and the procedures already in place to limit animal receipt to approved vendors and embryo rederivation, MNV was designated as an unacceptable pathogen for Facility 3. Depopulation of some colonies and testing and culling of others in that facility are underway.

The role of MNV as a confounding variable in experimental studies of mice remains unclear. We have informed and consulted with the investigators in our institute to determine their degree of concern. For some investigators, MNV was an acceptable pathogen in their animals. Other investigators, particularly those involved in immunologic studies of the innate immune system, required that their animals be maintained as MNV-free.

Although having all facilities free of MNV is desirable, this status currently is unfeasible given the time constraints of embryo rederivation; the widespread norovirus infection in Facilities 1, 2, 4, and 5; the high prevalence of MNV in colonies in the United States; the need for flexibility in moving mice between facilities and accepting mice from noncommercial sources; and the potential for viral persistence in immunocompetent mice. In fact, an extensive clean-up effort of all facilities is ill-advised until more is known about the spread of MNV and the ability to decontaminate a facility and maintain MNV-free colonies successfully.

Because MNV is widespread and could be easily introduced into an animal facility, maintenance of MNV-free animal rooms will require constant vigilance and monitoring of sentinel mice. The investigators at our institution have been informed that contamination remains a high risk, and decontamination may require eradication of infected mice and embryonic rederivation of mouse strains. Our future studies will continue to address the epidemiology and natural history of murine noroviruses so that effective prevention and control measures can be developed for our institute. Until then, routine sentinel testing for MNV is not performed in our facilities in which MNV is considered an acceptable contaminant.

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