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Prevention of Murine Norovirus Infection in Neonatal Mice by Fostering

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Murine norovirus (MNV) causes subclinical chronic infections in adult immunocompetent mice and is endemic in many mouse colonies. The susceptibility of neonatal mice to MNV infection was investigated. Intestinal homogenates from Swiss Webster (SW) mice inoculated orally with MNV-L on postpartum days (ppd) 1 to 3 were negative for MNV by RT-PCR at postinoculation days (pid) 3 and 7. In contrast, 69% of intestinal homogenates prepared on pid 3 and 7 from mice inoculated orally at ppd 5 to 8 were MNV-positive by RT-PCR. Because only mice 10 d of age or older were infected by contact with infected dams, a study was performed to determine whether fostering of neonatal mice from MNV-infected to MNV-naïve dams could be effective at preventing infection of neonatal mice. Four litters each of 1-, 2-, 4-, or 6 d-old mice from MNV-L– infected dams were transferred to naïve dams with similar-aged litters and vice versa. On ppd 21, feces from all MNV-infected dams and litters transferred to them were MNV-positive. In contrast on ppd 21, feces from all MNV-naïve dams and litters transferred to them were MNV-negative. Fostering of 2-d-old mice from 5 of 5 MNV-C–, 5 of 6 MNV-D–, and 7 of 8 MNV-G– infected dams onto MNV-naïve dams prevented MNV infection of the foster mice. In the 2 litters where MNV was detected, dams were infected within 7 d of transfer, suggesting that the neonatal mice had served as fomites. In summary, fostering was effective at preventing MNV infection in 33 of 35 litters of neonatal mice. Precautions to prevent transmission of virus on the surface of neonatal mice to foster dams could increase the efficiency of the fostering process.

Abbreviations: MNV, murine norovirus; pfd, post-fostering day; pid, post-inoculation day; ppd, postpartum day

AALASJournal of the American Association for Laboratory Animal ScienceSPECIAL TOPIC OVERVIEW200700010447May 20083Noroviruses (formerly called Norwalk-like viruses) infect humans, cows, pigs, and mice.[3,6] These viruses are emerging human pathogens that cause more than 90% of all epidemic viral gastroenteritis outbreaks in the United States.[8] Although seroprevalence for noroviruses in herds of swine and cattle ranges from 4% to 99%, disease is infrequent.[9,31] In contrast, human noroviral disease occurs within 48 h of exposure and is characterized by diarrhea, nausea, vomiting, and abdominal pain that lasts for 1 to 5 d.[17] People of all ages are susceptible to human noroviruses, but elderly persons, renal transplant patients, and those undergoing immunosuppressive therapy are at risk for more severe disease. Human noroviruses are readily spread by person-to-person contact, ingestion of contaminated food or water, or contact with contaminated surfaces.[8] Norovirus epidemics occur in many community settings, including nursing homes, childcare centers, schools, camps, hospitals, cruise ships, disaster shelters, and military bases.[16] The high prevalence of human norovirus infections is facilitated by the fact that only a few virus particles are necessary to initiate infection and by the high stability of the virus in the environment despite disinfection attempts.[4,22,30] In addition, there are more than 100 human norovirus strains in 3 genogroups, and elicited immune responses usually are not protective against infection with new strains of norovirus.[36]

Until recently, norovirus research was hampered by the failure of these viruses to grow in cultured cells and the lack of a small animal model. Murine norovirus (MNV) was identified in 2002 as a new mouse virus, and it is the first norovirus to be grown in cell culture.[18,34] MNV-1 causes a subclinical infection without remarkable tissue pathology in immunocompetent mice, and infection is limited to the intestines, liver, spleen, lymph nodes, and lungs.[14,18,24] Duration of infection ranges from less than 3 d to greater than 5 wk and appears to be dependent on the passage history of the viral inocula.[14,18] In contrast, MNV-1 caused a disseminated infection that included necrosis of the spleen, liver, lung, brain and intestines in mice with deficient innate immune responses (Stat1$^{-/-}$, Rag2$^{-/-}$/Stat1$^{-/-}$, Pkr$^{-/-}$/Stat1$^{-/-}$ and IFN$\alpha\beta\gamma$R$^{-/-}$ mice).[18] Intracerebral inoculation with MNV-1 was fatal in all STAT$^{-/-}$ mice by pid 7, whereas more than 60% of mice survived until pid 7 after intranasal or oral inoculation with MNV-1.[18]

More than 60 additional isolates of MNV have been identified and sequenced during the last few years, and they comprise a single genogroup and a single serotype.[28] None of these additional MNV strains has been reported to cause clinical disease in immunocompetent mice.[13,23,28] Similar to MNV-1, MNV strains WU11, WU12, WU23, and WU25 have been reported to cause diarrhea in mice with deficient innate immune responses (OT1/Rag1$^{-/-}$/IFNγR$^{-/-}$, OT2/ Rag1$^{-/-}$/IFNγR$^{-/-}$ and β_2M$^{-/-}$ mice),[28] and endemically infected 2- to 3-mo-old Rag1$^{-/-}$/IFNγR$^{-/-}$, OT1/Rag1$^{-/-}$ /IFNγR$^{-/-}$, OT2/ Rag1$^{-/-}$/IFNγR$^{-/-}$, and Rag1$^{-/-}$/Stat1$^{-/-}$ mice have been reported to have ruffled fur, hunched backs, and weight loss.[32]

Two recent studies reported the presence of MNV antibodies in 22% of sera tested and MNV RNA in feces of 64% of mouse strains tested, making MNV one of the most prevalent viruses in contemporary mouse colonies.[13,23] Because MNV can cause disease in immunodeficient mice, replicates in macrophages and dendritic cells in the spleen and liver, and can persist in mesenteric lymph node, immunologic studies could be affected adversely by MNV infection.[24,32,34] Therefore, methods for elimination of MNV from mouse facilities need to be developed.

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Because MNV causes persistent infections with shedding in feces for more than 1 mo,[3] efforts to let the infection die out most likely would be unsuccessful. MNV is stable between pH 2 and 10 and remains infectious for at least 7 d at room temperature in fecal material,[4] making decontamination efforts difficult and the risk of spread of MNV by fomites high. In addition, like with most noroviruses, very few viral particles are thought to be needed to initiate an MNV infection.[22] One method that has been successful at eliminating enteric infections from colonies of mice endemically infected with *Helicobacter hepaticus*, murine rotavirus, and mouse hepatitis virus is fostering of neonatal mice from infected to uninfected dams.[5,20,27,29,33] In the current study, the efficacy of fostering of neonatal mice from MNV-infected to MNV- naïve dams to prevent transmission of MNV to neonatal mice and the susceptibility of neonatal mice to MNV infection were determined.

Materials and Methods

Mice. Untimed pregnant or 4-wk-old female Swiss Webster mice (Tac:[SW]) were obtained from Taconic (Germantown, NY). On arrival at the destination institution, mice were seronegative for *Ectromelia* virus, murine rotavirus, lymphocytic choriomeningitis virus, mouse hepatitis virus, mouse parvovirus, minute virus of mice, murine norovirus, pneumonia virus of mice, reovirus, Sendai virus, and *Mycoplasma pulmonis* and were free of bacterial and parasitic infections. Mice were housed in an animal room with a negative pressure differential relative to the corridor, a 12:12-h light:dark cycle, and 10 to 15 air changes hourly. Mice were housed in filter-top static isolation caging on sterilized corncob bedding and were fed sterilized rodent chow (diet 5010, Purina Mills International, St Louis, MO) and hyperchlorinated water ad libitum by water bottle. Cages were changed weekly in a class II biosafety cabinet within the animal room, and caging and waste were autoclaved. All animal care and experimental procedures were approved by the Yale Animal Care and Use Committee and were in accordance with all federal policies and guidelines governing the use of vertebrate animals.

MNV stocks. Fecal samples were collected from 63 clinically normal, genetically engineered mice. Each fecal pellet was homogenized in 500 µl PBS, and total RNA was isolated from 50 µl of the homogenate by using RNeasy kits (Qiagen, Valencia, CA) according to the manufacturer's instructions. RT-PCR was performed by using the Superscript One-Step RT-PCR System (Invitrogen, Carlsbad, CA) and primers specific for the MNV capsid gene (MNV5662: GGC CGC CTT CTT TCT AAG CC and MNV 6507: GGA ACA CCC TGA CTG GGC AA). Primers were obtained from the WM Keck Foundation Biotechnology Resource Laboratory at Yale University (New Haven, CT). The reaction conditions for RT-PCR were: 30 min at 50 °C; 2 min at 94 °C; 40 cycles of 15 s at 94 °C, 30 s at 50 °C, 90 s at 68 °C; and 10 min at 68 °C. RT-PCR products were electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized by UV illumination. All RT-PCR assays included positive and negative controls. RNA extracted from 23 fecal samples produced an 865-basepair product. Sequencing of the products identified 13 isolates of MNV (A, B, C, D, E, F, G, H, J, K, L, M, and O; Genebank accession numbers, DQ269192 through DQ269204). Viral stocks and antisera were produced after oral inoculation of groups of five 4-wk-old SW mice with 20 µl/mouse of the fecal homogenate. Two mice in each group were euthanized by carbon dioxide overdose at postinoculation day (pid) 3, and intestines were harvested for viral stock preparation, and 3 mice were euthanized by carbon dioxide overdose at pid 30 for viral antisera collection. In addition, 10% intestinal homogenates were prepared in DMEM (Invitrogen) with 10% fetal bovine sera, aliquoted, and stored at –70 °C.

Comparison of the predicted amino-acid homologies within the hypervariable P2 domain of the capsid genes (147 amino acids) of 34 MNV strains (Genebank accession numbers: AY228235, DQ223041 through DQ223043, DQ269192 through DQ269204, DQ655664, DQ655666, DQ911368, EF531290, EF531291, EF650480, EF650481, EU004669, EU004671, EU004673 through EU004677, EU004678, EU004682, and EU004683) identified 5 groups of MNV strains in which each member of the group had at least 96% homology with at least 1 other member of the group. Group 1 included MNV-D, -K, -1, -5, -6, -CR5, -CR17, -CR18, and -Berlin05. Group 2 included MNV-C, -2, -3, -4, -CR6, and -Berlin06. Group 3 included MNV-B, -E, -F, and -G. Group 4 included MNV-J, -L, and -M. Group 5 included MNV-Berlin04, -M21-2, and M21-4. MNV-CR10 had high homology with members of both groups 1 and 5. Eight strains (MNV-A, -H, -O, -CR3, -CR4, -CR7, -WU24, and -WU26) had no more than 95% homology with all other strains. MNV-C, -D, -G, and -L were chosen for further study as representative members of groups 1 to 4.

Sample collection. A single fecal pellet was collected from the anus of each unanesthetized mouse while it was restrained gently; the pellet was frozen at –70 °C pending RT-PCR analysis. After euthanasia of mice by carbon dioxide overdose, spleen, kidney, liver, mesenteric lymph nodes, and intestines were collected aseptically and frozen at –70 °C for RT-PCR analysis or were fixed in 10% formalin or Bouin fixative. Paraffin sections of fixed tissues (thickness, 5 µm) were stained with hematoxylin and eosin for histology examination. Blood was collected postmortem by cardiocentesis.

Serology. Sera were tested for MNV antibodies by use of an immunofluorescent antibody assay. Immunofluorescent slides were prepared by infecting confluent monolayers of RAW 264.7 cells (obtained from American Type Culture Collection, Manassas, VA) with a filtered intestinal stock of MNV-D. Infected cells were harvested 24 h after inoculation, resuspended in Dulbecco minimal media with 10% fetal bovine sera (Invitrogen), and 10,000 infected or uninfected cells were placed on each spot of 12-spot slides. Cells were allowed to grow on the slides in a humidified chamber for 24 h, and then cells were fixed in acetone. Sera were tested at a dilution of 1:10, and binding of MNV antibodies was detected with fluorescein-conjugated goat antimouse antisera (Jackson Immunoresearch Laboratories, West Grove, PA)

Nucleic acid assays. Total RNA was isolated from feces or intestinal homogenates by use of RNeasy kits (Qiagen) according to the manufacturer's instructions. RT-PCR was performed by using the Superscript One-Step RT-PCR System (Invitrogen) and primers specific for the MNV capsid gene (MNV5033: GGA ACG CTC AGC AGT CTT TG and MNV5542: CAT TCA ACT CCC TCT TCT TG). The reaction conditions used for RT-PCR were as described above.

Fostering. Pregnant SW mice were observed twice daily to determine the time of birth for each litter. Litter sizes ranged from 6 to 19 pups, and at fostering litter sizes were reduced to a maximum of 12 neonatal mice per dam. At 24 to 36 h (postpartum day [ppd] 1), 48 to 60 h (ppd 2), 96 to 108 h (ppd 4) or 144 to 156 h (ppd 6) a cage containing an MNV-infected dam with litter and a cage containing a MNV-naïve foster dam with litter were placed in a class II biosafety cabinet. The litter was removed from the foster dam and placed in a plastic weight boat. A fecal sample was collected from the foster dam to confirm

that she was not infected with MNV. Age-matched neonatal mice were removed from the MNV-infected dam and were transferred onto the nest in the foster dam's cage, with care to not transfer bedding, nesting material, or fecal material from the cage containing the MNV-infected dam. A fecal sample was collected from the MNV-infected dam for RT-PCR analysis to confirm that she was shedding MNV. The neonatal mice from the foster dam, temporarily held in the weigh boat, were placed on the nest in the MNV-infected dam's cage. Exterior cage surfaces were disinfected with 1:5:1 Clidox (Pharmacal, Naugatuck, CT), and cages were returned to the cage rack. The exterior of the handler's gloves and the work surface of the cabinet were disinfected with Clidox, and the fostering procedure was repeated on additional sets of cages.

Statistics. Fisher Exact Probability Tests were performed by using http://faculty.vassar.edu/lowry/fisher.html.

Results

Infection of neonatal mice with MNV. In an effort to generate intestinal stocks of several MNV strains, 5 litters of neonatal SW mice (56 mice) and their dams were inoculated orally on ppd 1 or 2 with 10 µl of fecal homogenates containing MNV-A, -C, -D, -G, or -L. Ten pools of 4 to 6 intestines from neonatal mice harvested on pid 3 were all negative for MNV RNA by RT-PCR. In contrast, fecal and intestinal samples collected from the dams on pid 3 were all positive for MNV RNA by RT-PCR. To investigate at what age neonatal mice become susceptible to MNV, a pilot study was performed with 5 litters of SW mice. Neonatal mice and their dams were inoculated with MNV-G on ppd 3, 5, 7, or 8. All neonatal mice and dams remained healthy through pid 21 and did not show any clinical signs. Histologic examination of spleens, kidneys, livers, mesenteric lymph nodes, Peyer patches, small intestines, and large intestines from 6- to 21-d-old mice revealed no lesions (data not shown). Of the 21 neonatal mice inoculated on ppd 3, none (0 of 15) of the intestines tested on ppd 6 or 10 were positive for MNV RNA by RT-PCR, whereas feces and sera collected from the 3 dams and from 6 mice at weaning (ppd 21) were positive for MNV by RT-PCR and serology. Of the 34 neonatal mice inoculated on ppd 5, 7, and 8, 69% (11 of 16) of intestines tested were RT-PCR–positive for MNV at ppd 8 to 14 and 94% (17 of 18) of feces tested on ppd 17 or 21 were RT-PCR–positive for MNV. Therefore, neonatal mice were resistant to MNV infection after oral inoculation on ppd 1 to 3, but susceptibility to infection after oral inoculation developed by ppd 5. Because MNV infection was not detected on ppd 10 in mice inoculated on ppd 3, it appears that indirect infection of neonatal mice by contact with MNV-infected dams does not occur before ppd 11.

Fostering of neonatal mice from MNV-L–infected mice to MNV-naive dams. Sixteen pregnant SW mice were inoculated orally with MNV-L 2 to 5 d prepartum. Four litters of neonatal mice from MNV-L–inoculated dams were fostered to MNV-naïve dams on ppd 1, 2, 4, and 6 to determine the age range of neonates for which fostering could be used to generate MNV-free mice. Conversely, 4 litters of neonatal mice from MNV-naïve dams were fostered to MNV-inoculated dams on ppd 1, 2, 4, and 6. As expected, feces from all 16 MNV-inoculated dams were positive by RT-PCR for MNV RNA on the day of fostering, postfostering day (pfd) 7, and at the time of weaning (ppd 21), whereas feces from all 16 MNV-naïve foster dams were negative by RT-PCR for MNV RNA on the day of fostering, pfd 7, and at the time of weaning (ppd 21). All intestinal samples from neonatal mice born to MNV-infected dams (0 of 16) tested on pfd 7 were negative for MNV RNA (Table 1), indicating that MNV had not been transmitted from the dams to the neonatal mice prior to fostering on ppd 1 to 6. In addition, MNV RNA was not detected in intestines from 12 neonatal mice culled from MNV-positive dams on ppd 2 through 6 (data not shown). Fostering of neonatal mice to MNV-naïve dams was successful at preventing MNV-L infection, in that RT-PCR of all fecal RNA samples tested at ppd 21 and 45 (0 of 46) from mice fostered onto MNV-naïve dams were negative for MNV RNA, and sera from these mice were negative for MNV antibodies at ppd 45 (0 of 40; Table 1). All intestinal samples from neonatal mice fostered to MNV-infected dams (0 of 22) tested pfd 7 were negative for MNV RNA (Table 1), showing that MNV was not transmitted effectively to neonatal mice from the dams before ppd 13. Fostering of neonatal mice to MNV-L infected dams eventually resulted in infection of all foster mice, as MNV RT-PCR of the majority of feces tested at ppd 21 (16 of 18) and all feces tested at ppd 45 (8 of 8) were positive for MNV RNA, and sera from these mice were positive for MNV antibodies at ppd 45 (40 of 40; Table 1). None of the mice that became infected with MNV-L showed any clinical signs, and histologic examination of spleens, kidneys, livers, mesenteric lymph nodes, Peyer patches, small intestines, and large intestines from these mice revealed no lesions. These data indicate that transmission from MNV-infected dams did not occur in neonatal mice younger than 13 d but readily occurred in mice prior to weaning on ppd 21.

Fostering of neonatal mice from MNV-C–, -D–, and -G–infected mice to MNV-naive dams. MNV-L and other MNV strains in group 4 differ from many MNV strains in that attempts to culture them in vitro were unsuccessful (data not shown). To determine whether this difference in vitro might indicate that differences also exist in vivo, a second fostering experiment was performed to determine whether fostering of neonates from dams infected with other MNV strains to MNV-naïve dams could prevent MNV infection of neonatal mice. A single time point for fostering (ppd 2) was chosen. Twenty-seven untimed pregnant SW mice were inoculated orally 2 to 9 d prepartum with MNV-C, -D or -G, representative strains from groups 1 to 3. Nineteen litters of neonatal mice from MNV-inoculated dams were fostered to naïve foster dams on ppd 2. Other MNV-inoculated adult females either did not deliver litters, or uninoculated adult females did not deliver litters in time for them to be used as foster dams. In addition, 7 litters of neonatal mice from MNV-naïve dams were fostered to MNV-inoculated dams on ppd 2. Feces from all 19 MNV-inoculated dams were positive by RT-PCR for MNV RNA on the day of fostering, whereas feces from all 19 MNV-naïve foster dams were negative by RT-PCR for MNV RNA on the day of fostering. Most litters (17 of 19) of neonatal mice fostered from MNV-C–, -D–, or -G-inoculated dams to MNV-naïve dams remained free of MNV infection in that RT-PCR of fecal RNA tested at ppd 21 and 35 from mice fostered onto MNV-naïve dams were negative for MNV RNA, and sera from these mice were negative for MNV antibodies at ppd 21 or 35 (Table 2).

One litter of 12 neonatal mice fostered from an MNV-D–inoculated dam became infected. The dam to which these neonatal mice were fostered to became infected within the first week of fostering, as feces from this mouse were MNV-positive by RT-PCR on pfd 7. In addition, 1 litter of 10 neonatal mice fostered from an MNV-G–inoculated dam became infected. The dam to which these neonatal mice were fostered to became infected shortly after fostering, because feces from this mouse were MNV-positive by RT-PCR on pfd 2. The rapid infection of these 2 foster dams, during the time period in which neonatal mice remain free of infection, indicates that the neonatal mice

Table 1. Fostering of mice from MNV-L–infected dams

Age at fostering (d)	MNV status of foster dam	No. of mice fostered	Intestinal RT-PCR at pfd 7[a]	Fecal RT-PCR at ppd 21	Fecal RT-PCR at ppd 45	Serology at ppd 45
1	naïve	45	0/4 (4)	0/4 (9)	0/2 (2)	0/2 (10)
2	naïve	48	0/4 (4)	0/4 (10)	0/2 (2)	0/2 (10)
4	naïve	40	0/4 (4)	0/4 (9)	0/2 (2)	0/2 (10)
6	naïve	48	0/4 (4)	0/4 (10)	0/2 (2)	0/2 (10)
1	infected	48	0/4 (4)	4/4 (5)	2/2 (2)	2/2 (10)
2	infected	48	0/4 (4)	4/4 (4)	2/2 (2)	2/2 (10)
4	infected	48	0/4 (8)	4/4 (5)	2/2 (2)	2/2 (10)
6	infected	48	0/4 (6)	3/4 (4)	2/2 (2)	2/2 (10)

pfd, postfostering day; ppd, postpartum day
$P < 0.0001$ (Fisher Exact Probability Test) when fostering of litters of neonatal mice to MNV-naïve dams was compared with fostering of litters of neonatal mice to MNV-L-infected dams.
[a]No. of litters of mice positive for MNV-L RNA by RT-PCR or positive for MNV antibodies by IFA / no. of litters tested (no. of mice tested)

Table 2. Fostering of 2-d-old mice from MNV-C, -D, or -G–infected dams

Status of original dam	Status of foster dam	No. of litters (no. of mice fostered)	Fecal RT-PCR at ppd 21[a]	Fecal RT-PCR at ppd 35	Serology at ppd 21	Serology at ppd 35
MNV-C	naive	5 (49)	0/5 (11)	0/5 (14)	0/4 (20)	0/5 (23)
MNV-D	naive	6 (58)	1/5 (15)	1/6 (16)	1/5 (22)	1/6 (28)
MNV-G	naive	8 (89)	1/7 (17)	1/8 (18)	1/7 (37)	1/8 (40)
naive	MNV-C	3 (27)	3/3 (4)	3/3 (9)	3/3 (3)	3/3 (9)
naive	MNV-D	2 (20)	2/2 (3)	2/2 (8)	1/1 (2)	2/2 (8)
naive	MNV-G	2 (20)	2/2 (3)	2/2 (9)	1/1 (1)	2/2 (9)

ppd, postpartum day
$P < 0.0001$ (Fisher Exact Probability Test) when fostering of litters of 2-d-old mice to MNV-naïve dams was compared with fostering of litters of 2-d-old mice to MNV-infected dams.
[a]Number of litters of mice positive for MNV RNA by RT-PCR or positive for MNV antibodies by IFA / no. of litters tested (no. of mice tested)

may have served as fomites. The dams probably acquired the infection from grooming the neonatal mice, and subsequently the neonatal mice became infected via ingestion of virus-laden feces shed by the dam. Fostering of mice to MNV-C–, -D–, or -G-infected mice resulted in infection of all neonatal mice as MNV RT-PCR of all (36 of 36) of feces tested at ppd 21 and 35 were positive for MNV RNA, and sera from these mice were positive for MNV antibodies at ppd 21 and 35 (32 of 32; Table 2). All mice that became infected with MNV-C,-D, or -G did not show any clinical signs, and histologic examination of spleens, kidneys, livers, mesenteric lymph nodes, Peyer patches, small intestines, and large intestines from these mice revealed no lesions.

Discussion

Neonatal mice are generally more sensitive than weanling or adult mice to disease caused by enteric viruses. For example, intragastic inoculation of 1- to 14-d-old CD1 mice with murine rotavirus results in infection with diarrhea in the majority of inoculated mice, whereas inoculation of 15- to 20-d-old mice results in a sporadic asymptomatic infection, and inoculation of weanling and adult mice does not result in infection.[35] Oral inoculation of 1- to 7-d-old CD1 or BALB/cByJ mice with MHV-Y results in infection leading to severe enterocolitis with high mortality, whereas inoculation of 2-, 3-, and 12-wk-old CD1 or BALB/cByJ mice with MHV-Y results in subclinical mild enteritis.[1-3] In contrast, clinical signs were not observed in neonatal SW mice inoculated on ppd 1 through 8 with MNV-C, -D, -G, or –L, in agreement with a recent study that reported that 6 d-old 129S6/SvEvTac mice inoculated intragastically with MNV-1 did not develop diarrhea, and weight loss was not induced.[24] Even though clinical signs did not occur in neonatal mice or in dams after oral inoculation with MNV, infection as measured by the presence of MNV RNA in the intestine did occur—but only in mice 5 d of age or older. Further studies will be necessary to determine the mechanism of the resistance of 1- to 3-d-old mice to MNV, but it most likely is related to developmental changes occurring in the intestine and associated lymphoid tissues. Because antigen-presenting cells (macrophages and dendritic cells) are targets of MNV infection and because dendritic cells are essentially absent in several lymphoid tissues of 1 d old mice and are present only in very low numbers in 1-wk-old mice, the lack of this population of susceptible cells may contribute to the resistance of neonatal mice to MNV infection.[6,34]

Infection of neonatal mice by contact with infected dams, and their waste, occurred in mice after ppd 14, with almost all mice positive for MNV RNA in the feces by ppd 17. The timing of the development of susceptibility to MNV infection via natural routes coincides with several major maturational changes in the intestine of mice, including the cessation of nonselective and selective uptake of macromolecules (antibodies), upregulation of expression of enzymes necessary for the digestion of the carbohydrates in solid food, downregulation of lactase, changes in the glycosylation pattern of surface carbohydrates on brush-border membranes, and colonization by an 'adult' intestinal microflora.[10,11,26] These maturational changes indirectly affected the susceptibility of mice to MNV, as they prepared the mouse for the digestion of solid food. Mice in the current study began ingesting solid food around ppd 16 and were assumed to also begin ingestion of fecal material containing MNV, because mice typically become coprophagic at ppd 17 to 18.[7] Coprophagy appears to be the primary means of MNV transmission to young mice.

The presence of anti-MNV maternal antibodies did not prevent transmission of the virus from the dams to the neonatal mice. Antibodies to MNV-1 were detectable on pid 7 in 20% of experimentally infected mice and in the majority of infected mice by pid 14.[14,24] In the present study, pregnant mice were inoculated with MNV 2 to 9 d prepartum. Because most of the dams likely lacked antibodies to MNV when they gave birth, maternal antibodies probably played little, if any role, in the lack of infection in neonatal mice prior to fostering. All neonatal mice housed with MNV-infected dams should have acquired maternal antibodies by ppd 16, when ingestion of MNV-laden fecal material started. All neonatal mice housed with MNV-infected dams were both seropositive and positive for MNV RNA in there feces by ppd 21, indicating that maternally transferred antibodies did not protect against MNV infection. The persistent nature of the MNV infection in the face of a robust antibody response indicates that MNV antibodies also are insufficient to clear the virus in the dams.

The fostering procedure was designed to be as simple as possible. The procedure included several steps that differed from published fostering protocols and which may have resulted in the infection of 2 of the foster dams. Because mice were housed in filter-top cages, cages were only opened in the biosafety cabinet, all cages were disinfected prior to removal from the cabinet, and soiled cages were not dissembled in the animal room, it was not considered necessary to house MNV-infected and MNV-naïve dams in separate locations, as had been done in fostering protocols for other enteric murine infectious agents.[27,29,33] In addition, the fostering protocol used involved having a cage housing an MNV-infected dam and a cage housing a MNV-naïve dam open at the same time in the biosafety cabinet. Human noroviruses generally are not considered to be spread via an airborne route, though aerosol droplets of vomitus have been reported to be a possible source of infection.[21] Therefore the infection of the foster dams likely did not occur by transfer of virus in the air from the cage housing the MNV-infected dam to the MNV-naïve foster dam during the few minutes it took to transfer the neonates.

Lastly, the fostering protocol used did not include disinfection of the neonatal mice prior to transfer. Therefore, the neonatal mice may have served as fomites, and MNV present on the skin of neonatal mice appeared to be the most likely source of infection for the 2 MNV-naïve foster dams that became infected within a week of fostering. The transmission of MNV on the skin of neonatal mice in 2 litters to 2 naïve foster dams highlights the highly infectious nature of MNV. Like human noroviruses, MNV seems to require only a few viral particles to initiate infection, and therefore fomite-based transmission can readily occur after contact with a surface contaminated with a small amount of virus.[22] Fomite-based transmission of noroviruses is facilitated by the stability of the virus at room temperature and its relative resistance to several disinfectants.[4,30] Several fostering protocols for other viruses and bacteria have included disinfection of neonates with disinfectants such as Virkon or iodine.[19,33] To decrease the failure rate (5.7%) of the fostering process, disinfection of neonatal mice with an antimicrobial product effective against human noroviruses,[30] such as Virkon, could be added to the procedure. Alternatively for large litters, neonatal mice could be fostered onto 2 MNV-naïve dams.

In this study, foster dams did not display signs of rejection of the neonatal mice, possibly because outbred dams are known for their superior nurturing abilities.[25] In a previous study in which genetically modified mice on a C57BL background were disinfected prior to fostering to outbred foster dams, signs of maternal rejection of the fostered mice and potential hypothermia were observed.[33] Therefore if disinfection is included in the fostering process, care should be taken to mitigate these potential problems. In a second study, after signs of maternal rejection were observed when BALB/c neonatal mice were fostered to C57BL/6 foster dams, rejection was mitigated by covering the mice to be fostered with scented talc powder, mixing the mice from the donor and recipient litters prior to transfer, and (in a few cases) return of a few of the dams own pups back into her cage.[27] Dams in the current study were left in their home cages with the original bedding and nesting material, and no effort was made to alter the scent of the foster mice. Scent altering was unnecessary in this study to induce good maternal care of the fostered mice but might be necessary if the strain of foster dam used is less nurturing than the SW dams or if neonatal mice are treated with strongly scented disinfectants prior to fostering.

In the present study, most of the mice fostered on ppd 1, 2, 4, and 6 remained free of MNV. Several studies have investigated the time frame during which fostering is effective at preventing *Helicobacter hepaticus* infection of neonatal mice. One study indicated that fostering of neonatal mice from genetically engineered mice on a C57BL/6 background naturally infected with *H. hepaticus* to C57BL/6 dams within 24 h of birth prevented *H. hepaticus* infection.[29] A second study investigated the efficacy of fostering of neonatal mice from experimentally infected C57BL/6 dams onto BALB/c dams at later times (ppd 1 through 14) and found that for only the group of mice fostered on ppd 1, all remained free of *H. hepaticus*.[27] In contrast, a third study indicated that fostering of C57BL/6 neonatal mice to BALB/c dams on ppd 1 to 4 d prevented *H. hepaticus* infection, but only if the sire was not present in the cage.[5] Use of medicated feed in conjunction with fostering within 24 h of birth seems to be the most effective method of preventing the transmission of *H. hepaticus* and fur mites to neonatal mice.[15,19] Several studies have investigated the effectiveness of fostering within 36 h of birth for the prevention of the enteric viruses mouse hepatitis virus, mouse parvovirus, murine rotavirus, and Theiler encephalomyelitis virus and found that fostering is effective at preventing all but Theiler encephalomyelitis virus infection.[12,20,33] The longer time period for which fostering is effective at preventing MNV infection, relative to other enteric agents, probably results from the resistance of neonatal mice to MNV infection.

In conclusion, fostering is a relatively simple process that can be used to eliminate multiple enteric pathogens from a population of mice. If the goal is to eliminate MNV only, then fostering can be performed any time before ppd 6. However, if the goal of the fostering process is to eliminate multiple agents from a population of mice, then fostering should be performed within 24 h of birth and the use of medicated feed should be considered if nonviral agents are to be eliminated.

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