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Antibody Titers and Seroconversion Kinetics of Outbred Swiss and Heterozygous Nude Soiled-bedding Sentinels for Murine Norovirus and Mouse Hepatitis Virus

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Sentinel animals remain a common means of evaluating rodent health in research colonies. An evaluation of our sentinel program revealed that using Crl:CD1(ICR)-Elite (CD1-E) mice was expensive, occasionally disrupted by limited supply, and minimally responsive to the 3Rs. This evaluation prompted us to explore the use of CRL:NU-*Foxn1*$^{nu/+}$ (Het-nude) mice as soiled-bedding sentinel (SBS) animals. Het-nude mice are a byproduct of breeding outbred athymic nude mice and are reared in isolators, with similar health status as CD1-E. Het-nude mice have a thymus, but may have smaller thymic size and fewer bone marrow stem cells than do wildtype controls, suggesting that Het-nude mice might not be immunologically normal. This study compared the antibody titer and seroconversion kinetics of Het-nude and CD1-E SBS to murine norovirus (MNV) and mouse hepatitis virus (MHV). Het-nude and CD1-E female SBS (n = 22 mice of each stock) were housed continuously on soiled bedding collected from MNV-positive or MNV- and MHV-positive colonies at cage changes. Blood was collected for serology at 3, 9 and 12 to 19 wk after the start of soiled bedding exposure. Antibody titers to MNV or MHV did not differ significantly between Het-nude and CD1-E mice. A significant relationship was found between weeks of exposure and titer levels with an increase in titer over the testing period. This study supports the possible use of Het-nude mice as SBS, given that their antibody responses to MNV and MHV are equivalent to those of CD1-E mice.

Abbreviations: CD1-E, CRL: CD1(ICR)-Elite; Het-nude, CRL: NU-*Foxn1*$^{nu/+}$; ICR, Crl: CD1; MNV, Murine norovirus; MHV, mouse hepatitis virus; SBS, soiled-bedding sentinel

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Sentinel animals remain a mainstay of pathogen detection in colonies of rodents housed in IVC in research facilities, despite limitations in the sensitivity of sentinel testing and advances in environmental health monitoring technologies.[1,6,15,18,23] Detection of adventitious pathogens by using devices that filter exhaust air from IVC is significantly more sensitive for labile organisms in the environment or that transmit poorly through soiled bedding.[15,18,23,31] However, limitations of these methods can cause institutions to continue to use soiled-bedding sentinel (SBS) animals. One obstacle is that IVC systems were not originally designed for exhaust debris sampling. The flow of exhaust air within the rack and the design for filtration of exhaust debris heavily influence the ability to detect pathogens.[1] For institutions that do not use models of IVC systems shown to detect pathogens via exhaust air testing, this approach is not possible without the considerable expense of replacing existing equipment. Additional drawbacks to environmental sampling techniques include false-positive results from residual nucleic acids harbored in equipment and false negatives due to dilution or low prevalence of disease.[15,18,23] For institutions that continue to use SBS animals to monitor the health of their rodent colonies, the strategic selection of sentinel animals is key to maximizing sensitivity and supporting the 3Rs.

Multiple factors should be considered when choosing an appropriate sentinel strain or stock for rodent colonies.[6,9] The ideal sentinel has an immune system capable of mounting a detectible antibody response to the target pathogen after being exposed to an infectious dose in soiled bedding. The strain or stock should be susceptible to the pathogens that significantly affect the type of research being performed, and need to be of an appropriate age to be susceptible to the pathogen.[7,9] The sentinel's health status should exclude pathogens that would put research animals at risk during soiled-bedding transfer or cause false-positive results. Ideal sentinel animals are consistently available from vendors, have verifiable health status, are reasonably priced, and are produced in a manner that is responsive to the 3Rs. Our institution recently used these criteria to critically evaluate the sentinel mice we were using in our facilities.

At our institution, we traditionally used vendor-purchased, 3- to 4-wk-old, female outbred mice with a Swiss lineage, Crl:CD1(ICR), for our sentinel program. As our mouse colonies became more populated with immunocompromised animals and projects that relied on the exclusion of opportunistic bacteria, we shifted to outbred mice produced under microisolation conditions, such as CRL:CD1(ICR)-Elite (CD1-E) mice. Although the health status of these mice is reliable, their availability is often inconsistent, and they are expensive. To refine our sentinel

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program, we considered whether the use of female heterozygous nude mice, CRL:NU-*Foxn1*$^{nu/+}$ (Het-nude), as sentinels would provide the same health status, support equivalent health monitoring, and address availability and cost concerns. In addition, the use of Het-nude mice would support the spirit of the 3Rs, because they are a byproduct of nude mouse colonies.

Athymic nude mice (*Foxn1*$^{nu/nu}$) are commonly used for cancer xenograft studies. Production colonies of athymic nude mice are maintained by mating male athymic nude mice with female heterozygotes (*Foxn1*$^{nu/+}$).[28] Female mice must be heterozygous due to the poor lactation of female athymic nude mice, leading to high neonatal losses.[10,17] The mating scheme results in approximately 50% of homozygous offspring (athymic nude) and 50% heterozygous.[10,17] Other than for breeding purposes, there is no common use for the Het-nude genotype. Furthermore, commercial vendor breeding colonies maintain athymic nude mice in isolators, which exclude opportunistic microorganisms.

The excess isolator-raised Het-nude mice could be a viable alternative to other isolator-raised outbred sentinel mice. However, Het-nude mice reportedly may not be immunologically normal. The absolute and relative thymic weights are lower in Het-nude mice than in wildtype *Foxn1*$^{+/+}$, and the lower weight is related to a decrease in the lymphoid population.[2,21,25] In addition, Het-nude mice have fewer bone marrow stem cells and greater individual variability in the number of stem cells, compared with wildtype mice.[11] Het-nude mice produce serum IgM, IgG1, IgG2a, IgG2b, IgG3, and IgA.[19] Despite these features, some facilities currently use Het-nude mice as SBS animals. However, Het-nude mice have not been compared with other isolator-raised outbred sentinel mice. The current study sought to compare the antibody responses of CD1-E and Het-nude mice to soiled bedding from colony animals endemically infected with murine norovirus (MNV) and mouse hepatitis virus (MHV), given the institutional and broad prevalence of those viruses.

MNV and MHV are among the most prevalent infectious agents found in contemporary laboratory mouse colonies.[24] MNV is a nonenveloped, single-stranded RNA virus in the *Caliciviridae* family, and MHV is an enveloped, single-stranded RNA virus of the *Coronaviridae* family. Both organisms are transmitted via the fecal–oral route and can be transmitted effectively in contaminated soiled bedding. Due to its nonenveloped structure, MNV can remain infective in the environment for at least 14 d, whereas MHV has a much shorter time-span, losing its infectivity within 3 d.[3,16,27]

The effects of having these pathogens present in mouse colonies depends on the virus strain and host's genetic factors. MNV is considered to be relatively innocuous, causing asymptomatic infections that do not result in significant pathology in immunocompetent strains, but MNV may cause disease in certain immunodeficient strains, such as STAT1-null mice and those lacking interferon α, β, or γ.[20,30] In addition, MNV has been implicated in causing disruptions in mouse models of inflammatory bowel disease and atherosclerosis.[13,14] MHV infections are associated with variable clinical signs and pathology, depending on the age, strain, immunologic status of the mouse, and the tropism of the viral strain. Enterotropic strains are restricted to the intestines and mesenteric lymph nodes, whereas polytropic strains cause systemic infections and can localize in the intestines, liver, spleen, and brain.[3,5,12,26,29]

Years of quarterly SBS testing records indicate that various rodent colonies at our institution are endemically infected with MNV, MHV, or both. We hypothesized that isolator-raised outbred Swiss CD1-E and Het-nude mice both would seroconvert to 2 of the most common mouse microorganisms,[24] MNV and MHV, after exposure to soiled bedding for 3 mo.

Materials and Methods

Mice. For this study, 2 groups of CRL:NU-*Foxn1*$^{nu/+}$ (Het-nude) and CRL:CD1(ICR)-Elite (CD1-E) mice were exposed to soiled bedding several months apart. Group 1 consisted of 18 Het-nude and 18 female CD1-E mice to evaluate seroconversion to MNV. Within Group 1, 8 mice (4 of each stock) were negative controls that were not exposed to any soiled bedding or were exposed to soiled bedding from a population that was negative for MNV and MHV. Therefore, 28 mice (14 of each stock) were exposed to soiled bedding from MNV-positive populations in total. Group 2 consisted of 8 Het-nude and 8 CD1-E mice to evaluate seroconversion to MNV and MHV; all 16 of these mice were exposed to soiled bedding from MNV- and MHV-positive populations (no negative controls). Female mice were 3 to 4 wk old upon arrival at the facility and pair-housed with mice of the same stock. All mice were from Charles River Laboratories colonies that had tested negative for the following agents: Sendai virus, pneumonia virus of mice, MHV, minute virus of mice, mouse parvovirus, Theiler murine encephalomyelitis virus, reovirus, rotavirus, mouse adenovirus, polyoma virus, K virus, murine cytomegalovirus, mouse thymic virus, lymphocytic choriomeningitis virus, hantavirus, ectromelia, lactate dehydrogenase elevating virus, MNV, *Bordetella bronchiseptica*, *Corynebacterium bovis* and *C. kutscheri*, *Citrobacter rodentium*, *Helicobacter* spp., *Klebsiella oxytoca* and *K. pneumoniae*, *Pasteurella multocida* and *Rodentibacter pneumotropicus*, *Rodentibacter heylii, Proteus mirabilis*, *Pseudomonas aeuringosa*, *Salmonella* spp. *Staphylococcus aureus*, *Streptococcus pneumoniae*, β-hemolytic *Streptococcus* spp., *Streptobacillus moniliformis*, *Pneumocystis* spp., *Clostridium piliforme*, *Filobacterium rodentium*, *Mycoplasma pulmonis*, ectoparasites, endoparasites, enteric protozoa, and *Encephalitozoon cuniculi*. All animals were monitored daily by animal care staff.

Facility. All experimental procedures were approved by the Pennsylvania State College of Medicine IACUC; the institutional animal care program is AAALAC-accredited. SBS animals were housed in both conventional (12 mice of each stock, 24 mice total) and barrier (14 mice of each stock, 28 mice total) animal facilities. Conventional rodent rooms had static open-top cages (Max 75 polycarbonate, Alternative Design Manufacturing and Supply, Siloam Springs, AR) with corncob bedding (Teklad 7092-7097, Envigo, Madison, WI) and nesting material (Enviropaks, WF Fisher and Son, Branchburg, NJ). Mice were fed standard rodent chow (Teklad 2018, Envigo) and tap water ad libitum. Barrier rodent rooms had IVC (Max 75 polycarbonate, Alternative Design Manufacturing and Supply) at positive pressure to the room, with 60 air changes per hour. IVC are autoclaved as a unit, with the same corncob bedding and nesting as described for conventional rooms. Mice were provided with irradiated rodent chow (Teklad 2918, Envigo) and autoclaved tap water ad libitum. Conventional and barrier rooms were both maintained under a 12:12-h light:dark cycle (lights on at 0700; off at 1900). Both housing areas were maintained at a temperature range of 68 to 75°F (20 to 24°C) and a humidity range of 30 to 70%.

Soiled-bedding collection method. Soiled bedding was systematically collected from 10% to 20% of the colony cages on a rack during cage change (every wk for conventional housing, every 2 wk for barrier housing) and was added to sentinel cages for continuous exposure to soiled bedding. For IVC racks, one-half cup of soiled bedding was collected from each of 10 to 14 colony cages at each cage change. The soiled bedding was mixed

and split into 2 sterile IVC cages so that CD1-E and Het-nude mice were continuously exposed to the same soiled bedding, with newly created soiled bedding cages being introduced every 2 wk during the room cage change. The same procedure was performed for conventional open-top cages, except that one cup of soiled bedding was collected from each of 4 or 5 colony cages, and the cages were not sterile. Rooms chosen for the study had historically been positive for MNV, MHV, or both for at least the previous year, according to the sentinel monitoring program results for those rooms. Two mice of each stock (4 mice total) were not exposed to soiled bedding in a room that did not have MNV or MHV in the population. Two mice of each stock (4 mice total) were exposed to soiled bedding in a room that was negative for both MNV and MHV.

Sample collection and storage. Blood was collected for serology from all mice at 3 and 9 wk after beginning exposure to soiled bedding and at the end of the sentinel quarter (14 to 19 wk after soiled bedding exposure had been initiated for group 1 or 12 wk for group 2). For the 3- and 9-wk samples, mice were manually restrained, and a 5-mm lancet (Goldenrod, Medipoint, Mineola, NY) was used to collect about 20 µL of blood from the facial vein. The final sample was collected via cardiac puncture after respiration had ceased for at least 1 min after exposure to CO_2. Two mice (one CD1-E mouse from group 1 and another CD1-E mouse from group 2) were euthanized before the final blood collection time point due to imperforate vagina and nonspecific clinical disease, respectively. All blood samples were placed on an Opti-Spot card (IDEXX BioAnalytics, Columbia, MO) and stored at 4 °C until all samples could be submitted for analysis simultaneously. In addition, body, thymus and spleen weights were collected for all group 2 animals at the time of euthanasia. A veterinary pathologist performed complete gross necropsies on all group 2 mice.

Thymuses from group 2 mice were fixed for 24 h in 10% neutral-buffered formalin and then were soaked in 70% ethanol until they were paraffin-embedded according to routine protocols. Blocks were cut into 6-µm-thick sections and stained with hematoxylin and eosin. The cortex:medullary ratio was calculated to determine whether the relative sizes of the thymic cortex and medulla differed between stocks. To calculate the cortex:medullary ratios, the thymus was imaged at 40× original magnification, and the cortex and medulla were measured (in µm) at 10 randomly selected points scattered throughout both lobes, to decrease any lobe thickness variability associated with the embedding process.[8] Each of the 10 points was used to calculate a cortex:medullary ratio, with all 10 ratios averaged for each animal. Other measures of cellularity and relative degree of thymic age-related involution were measured through mean cortical lymphocyte density and apoptotic body density as well as medullary epithelial cell and apoptotic cell density. These cellular densities were each calculated by using 4 (original magnification, 400×) images obtained from the thymus slides.[8] Density was calculated as the number of cells per millimeter squared. All histomorphometry analyses were performed by a veterinary pathologist who was blind to groups. Images were captured by using an Eclipse E200 (Nikon Instruments, Tokyo, Japan) with an attached Infinity 2 camera and Infinity Analyze analysis software (version 7.0.920, Teledyne Lumenera, Nepean, Ontario, Canada).

Serology. For group 1 mice, antiMNV antibody levels were measured using the LX200 (Luminex. Austin, TX) system. Antibody was eluted from each dried blood spot sample and serially diluted 1:2. Microspheres coupled to purified native MNV or MNV VP1 protein were suspended by vortexing and sonication. Approximately 1500 microspheres in a total volume of 100 µL PBS-BSA (PBS, 1% bovine serum albumin, 0.05% sodium azide; Sigma–Aldrich, St Louis, MO) along with the test sample were added to each well of an AcroPrep 96-well filter-bottom plates (Pall, Port Washington, NY). Plates were covered and incubated for 60 min on an orbital shaker (400 rotations per minute) at room temperature in the dark. Each well was washed 4 times by adding 100 µL of PBS-BSA, shaking at 900 rotations per minute for 1 min, and then removing the fluid with a vacuum manifold. The microspheres were suspended in 100 µL of PBS-BSA containing $F(ab')_2$ fragment goat antimouse IgG biotin-conjugated secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA). The plate was covered and incubated for 60 min on an orbital shaker at 400 revolutions per minute at room temperature in the dark. Plates were washed 4 times as described above, and microspheres were suspended in 100 µL of fresh PBS-BSA containing streptavidin-phycoerythrin (Moss, Pasadena, MD.). The plate was covered and incubated for 30 min on an orbital shaker at 400 revolutions per minute at room temperature in the dark. Plates were washed 4 times; microspheres were suspended in 100 µL of fresh PBS-BSA and analyzed on the Luminex workstation. The median fluorescent intensity of 100 MNV purified virus and MNV VP1-coated microspheres was recorded; 3500 fluorescent units was used as the cutoff value for raw data. Virus antibody titers were then calculated from the raw data for analysis.

Group 2 serology samples were evaluated using a multiplex fluorescent immunoassay with 2 fluorescent beads for each viral agent. Beads are coated with whole or recombinant protein VP1. The values were reported as the median fluorescent value (MFV). The sample was considered positive when the MFV exceeded a previously determined baseline value (3 for purified MNV, 4 for recombinant MNV, and 2.5 for purified and recombinant MHV). Any discrepancies between multiplex fluorescent immunoassay results were confirmed as positive or negative by using an immunofluorescent antibody test. Purified virus MFV was used for statistical analyses because there were no statistically significant differences between purified and recombinant value results.

Statistics. MNV antibody titers from group 1 mice and group 2 median fluorescence values for MNV and MHV were compared between stocks and across time by using 2-way ANOVA and a multiple-comparisons posthoc test. Body weights for CD1-E and Het-nude mice were analyzed by using the Welch T-test. Splenic and thymic weights were standardized according to percentage of body weight then analyzed by using a Student *t* test (splenic weights) or Mann–Whitney test (thymic weights). Thymic histomorphometry parameters were analyzed by using Mann–Whitney (cortex:medullary ratio, lymphocyte density) or Welch *t* (cortex apoptotic bodies, medullary epithelial cells and apoptotic bodies) tests. All statistical analyses were performed by using Prism 8 (GraphPad Software, San Diego, CA). *P* values less than 0.05 were considered statistically significant for all tests.

Results

Serology. The negative controls (mice not exposed to soiled bedding and mice exposed to soiled bedding from an MNV- and MHV-negative population) did not have measurable antibodies throughout the experiment (data not shown). Two CD1-E mice (one from each group) were excluded from data analysis, because they were euthanized (due to imperforate vagina and nonspecific clinical signs, respectively) after the first blood collection.

The results from all mice exposed to soiled bedding from MNV-positive populations (20 CD1-E and 22 Het-nude) are shown in Table 1. Although experiments were performed several months apart, MNV seroconversion results were similar for groups 1 and 2. AntiMNV antibody titers or median fluorescence values were not significantly different between Het-nude and CD1-E mice (Figure 1 A, $P = 0.91$; Figure 1 B, $P = 0.16$; respectively). However, for all groups, a significant difference was detected between titers and week of exposure, with titers increasing over time (Figure 1 A, $P < 0.001$; Figure 1 B, $P < 0.0001$, respectively). After 3 wk of soiled-bedding exposure in MNV-positive rooms, 2 of 20 (10%) CD1-E mice and 5 of 22 (22.7%) Het-nude mice were seropositive for MNV. After 9 wk of continuous exposure to soiled bedding, 14 of 20 (70%) CD1-E mice and 19 of 22 (86.4%) Het-nude mice were seropositive for MNV. At necropsy, all (22 of 22, 100%) of the Het-nude mice were seropositive for MNV, but only 17 of 20 (85%) of the CD1-E mice were MNV-positive.

Many of the group 2 CD1-E and Het-nude mice were seropositive for MHV after 3 wk of continuous exposure to soiled bedding. Similar to MNV, a significant increase in MFV occurred over time (Figure 1 C, $P = 0.015$), but no difference in the MFV was found between CD1-E and Het-nude mice ($P = 0.14$). In total, 6 of the 7 (86%) CD1-E mice and 5 of the 8 (62%) Het-nude mice were seropositive at 3 wk after exposure. By week 9, 6 of the 7 (86%) CD1-E mice and 7 of the 8 (87%) Het-nude mice had seroconverted. The final mouse in the CD1-E and Het-nude cohorts did not seroconvert by necropsy, and the percentage positive values remained at 86% and 87%, respectively.

Body and thymic weights. No appreciable gross differences were detected between the group 2 CD1-E (Figure 2 A) and Het-nude (Figure 2 B) mice at necropsy. The descriptive statistics for body weight, thymic weight, and splenic weight are provided in Table 2. Group 1 and group 2 body and thymic weights were not significantly different, so values were combined for analysis. CD1-E mice were heavier than Het-nude mice (Figure 2 C, $P = 0.001$). The body weight (mean ± SEM) for CD1-E mice was 27.6 ± 1.3 g; that for Het-nude mice was 25.9 ± 0.6 g. No significant difference was found between relative thymic weights (calculated as a percentage of body weight) of Het-nude and CD1-E mice (Figure 2 D, $P = 0.08$). The mean, SEM, and 95% confidence intervals for thymic weights were 0.24 g, 0.02 g. and 0.2 to 0.27 for CD1-E mice and 0.27 g, 0.02 g. and 0.24 to 0.33 for Het-nude mice, respectively. Likewise, splenic weights as a percentage of body weight were similar between the group 2 cohorts ($P = 0.76$). The only gross lesion observed during sample collection was multifocal, minimal hepatic extramedullary hematopoiesis in a single group 2 CD1-E mouse.

Thymic histology. Histomorphologic analyses revealed 2 significant differences between CD1-E and Het-nude mice. Due to their larger cortex, Het-nude mice had significantly higher cortex:medullary ratios than did CD1-E mice (Figure 3 A through C, $P = 0.019$). The cortex:medullary ratio (mean ± SEM) was 1.9 ± 0.2 for Het-nude mice and 1.3 ± 0.1 for CD1-E mice. In addition, compared with CD1-E mice, Het-nude mice had a significantly higher cortical apoptotic body density (Figure 3 D, $P = 0.039$) without a difference in cortical lymphocyte density (not shown, $P = 0.072$). Apoptotic bodies were identified free and within macrophages (that is, tingible body macrophages). No differences were detected in medullary epithelial cell (not shown, $P = 0.86$) or medullary apoptotic body density (not shown, $P = 0.61$) between the 2 groups.

Table 1. Seroconversion results for CD1-E and Het-nude mice

		Time (wk) after exposure		
	n	3	9	12 to 19 (at necropsy)
MNV groups 1 and 2				
CD1-E	20	2/20 (10.0%)	14/20 (70.0%)	17/20 (85.0%)
Het-nude	22	5/22 (22.7%)	19/22 (86.4%)	22/22 (100%)
MHV group 2				
CD1-E	7	6/7 (85.7%)	6/7 (85.7%)	6/7 (85.7%)
Het-nude	8	5/8 (62.5%)	7/8 (87.5%)	7/8 (87.5%)

Data are given as no. of mice positive/ total no. of mice tested.
Mice were serologically negative for all other pathogens tested (results not shown). A total of 8 mice in group 1 ($n = 4$ each CD1-E Het-nude) were either not exposed to soiled bedding or exposed to soiled bedding from an MNV-negative population were not included in this table.

Discussion

This study demonstrated that isolator-reared female Het-nude and CD1-E mice are equivalent for use as SBS animals to detect MHV and MNV by seroconversion. All Het-nude mice and 85% of CD1-E mice in MNV-positive rooms seroconverted to MNV by the end of the study period. These seroconversion rates of the SBS animals to MNV at the end of the quarter is higher than what has been reported by others.[16,31] This higher seroconversion percentage may be due to a sampling effect. Rooms that historically had higher SBS seroconversion rates to MNV were chosen for the group 1 study. If we had studied all mouse rooms, sentinel mice would likely have had a lower seroconversion rate to MNV. Some SBS animals did not seroconvert to MNV until the last time point (12 to 19 wk), but just over 78% of both Het-nude and CD1-E SBS had seroconverted after 9 wk of exposure to soiled bedding. This study was designed to mimic the regular health monitoring program used at the institution; further studies could be done with controlled exposure of sentinels to MNV. The findings from our study and other reports[16,31] reveal that when soiled-bedding sentinels are used for health monitoring (for 3 mo or less, for example, quarantine) to detect MNV, facilities should consider additional diagnostics for MNV, such as direct testing of colony animals or fecal PCR from sentinel animals. In addition, this study revealed that the thymuses from Het-nude mice had larger cortices and more apoptotic lymphocytes than CD1-E mice.

Several reports have demonstrated that MHV is readily transmitted via the feces and that SBS animals will seroconvert when exposed to MHV.[4,6,27] In the current study, 85% to 87% of SBS animals seroconverted to MHV, with most animals seroconverting by 3 wk after exposure to soiled bedding. No difference in detection was identified between Het-nude and CD1-E SBS, and either stock can be used to detect MHV effectively. Limitations of this study are that the group used for MHV seroconversion was small and the experiment was performed in a facility with open-top cages. Additional studies may be warranted to confirm these findings and confirm seroconversion in facilities using microisolation caging.

The differences in thymic cortex:medullary ratio and number of apoptotic cortical lymphocytes without a significant difference in relative thymic weight between stocks provides some insight into thymus physiology. A larger cortex may indicate a greater immature or unstimulated lymphocyte population in the Het-nude mice, given that immature lymphocytes predominate

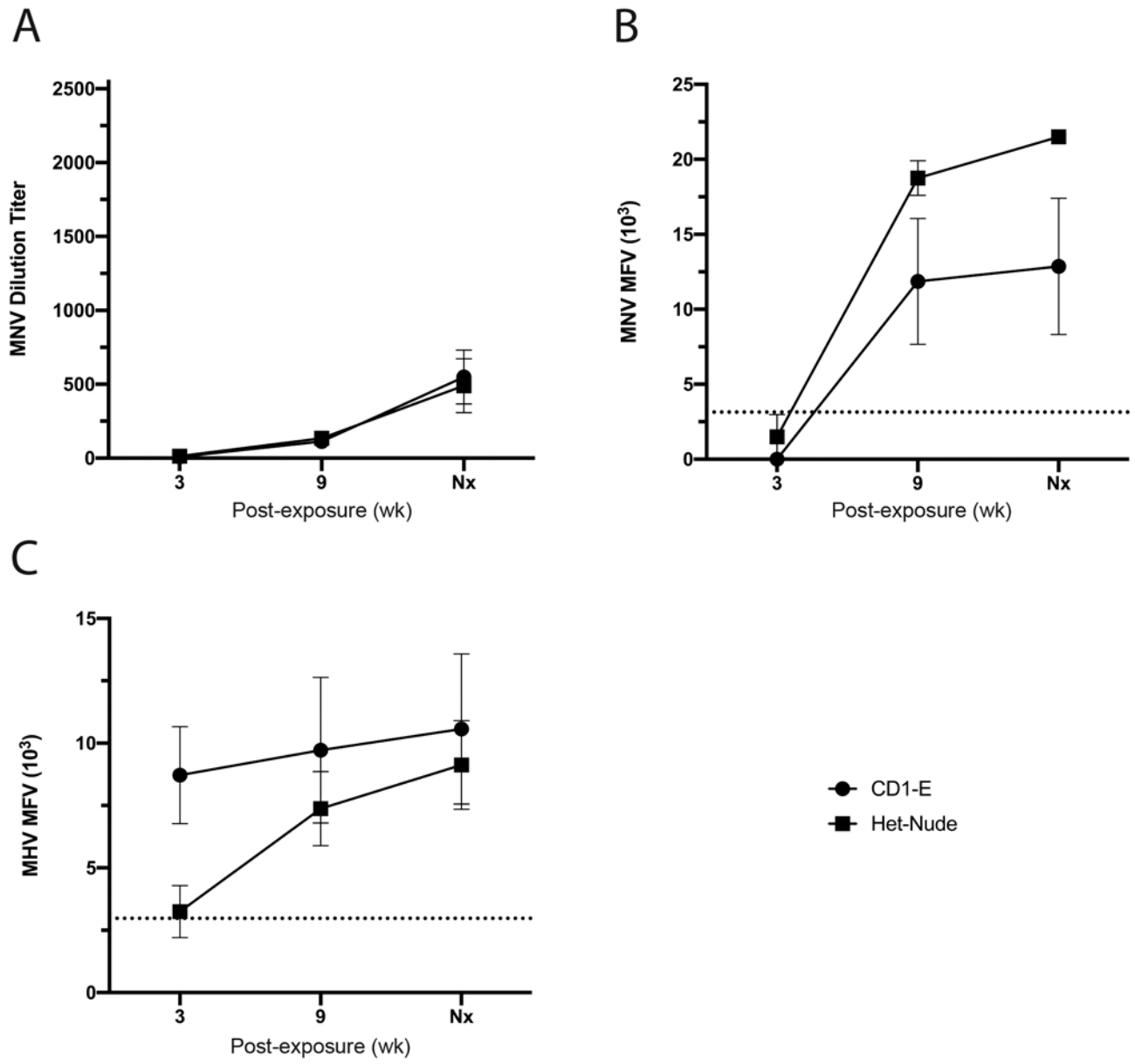

Figure 1. Antibody responses of heterozygous nude (Het-Nude) and CD1-Elite (CD1-E) mice after exposure to soiled bedding from colony mice known to be positive for murine norovirus (MNV) and hepatitis virus (MHV). (A) Group 1 and (B) group 2 Het-nude and CD1-E mice had detectable titers to MNV by week 9 after exposure to soiled bedding. There was a significant increase in MNV titers and median fluorescent value (MFV) across time points (A, $P < 0.001$; B, <0.0001), but there was no significant difference between CD1-E and Het-nude mice at each time point (A, $P = 0.91$; B, $P = 0.16$). C) The average MHV MFV for CD1-E and Het-nude mice was above the positive threshold by 3 weeks after exposure. Overall, there was a significant increase in MHV MFV across time points for both stocks (2-way ANOVA posthoc analysis, $P = 0.015$). Similar to MNV: there was no significant difference between CD1-E and Het-nude MFV at each time point ($P = 0.14$). Necropsy was performed between 14 and 19 wk after exposure to soiled bedding for group 1 and at 12 weeks for group 2. Error bars represent the SEM. Group 1: CD1-E, $n = 13$; Het-nude, $n = 14$. Group 2: CD1-E, $n = 7$; Het-nude, $n = 8$. The dotted line indicates the positive threshold for MFV, which was 3 for purified MNV, 4 for recombinant MNV, and 2.5 for both purified and recombinant MHV. The scale of the *y*-axis in panel A represents the full range of possible titers. Purified virus MFV are shown in panels B and C, because there were no significant differences between purified and recombinant value results. Nx, necropsy. MFV, median fluorescent value.

within the cortex, compared with more mature lymphocytes in the medulla.[22] The relative increase in apoptotic lymphocytes without an increase in cell density may suggest increased early activation and turnover of cortical lymphocytes with antigenic stimulation or endogenous steroid release from debilitation or stress.[8,22] We suspect the apoptotic lymphocytes may have been related to early antigenic stimulation because none of the Het-nude mice showed clinical signs of illness. Many times, lymphocyte density will increase with antigenic stimulation.[8] The histomorphometry we performed provided only a 'snapshot' of thymus development for these stocks. The increase in cortical apoptotic lymphocytes may have been present before mean cell density increased. Histomorphology, along with immunophenotyping at several ages and in conjunction with

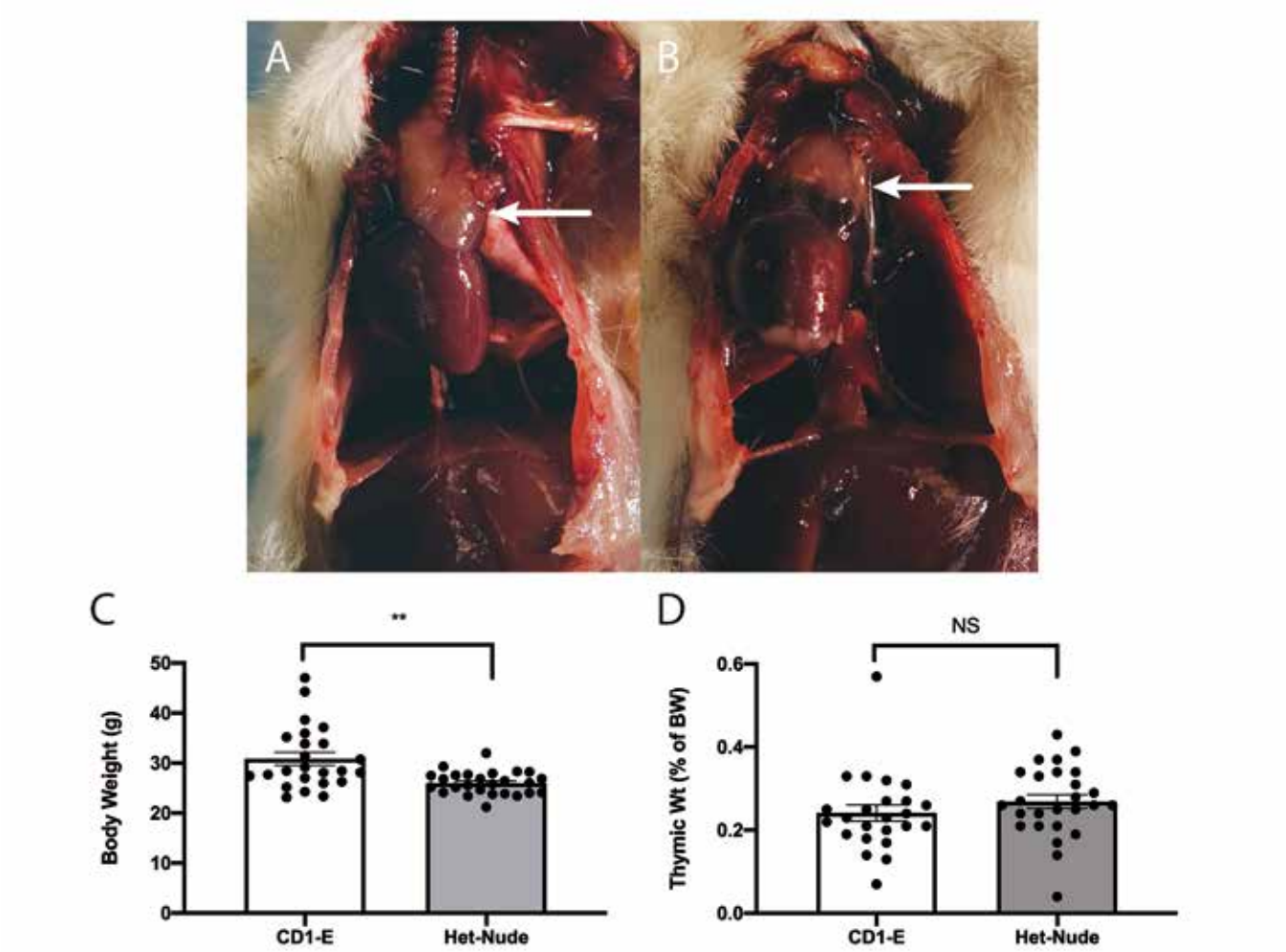

Figure 2. Gross appearance of thymus, body weight, and, and thymic weight for CD1-E and Het-nude mice. There was no difference in the gross appearance of the thymus between (A) CD1-E and (B) Het-Nude mice. (C) CD1-E mice weighed more (Welch *t*-test, $P = 0.001$) at necropsy compared with Het-nude mice. (D) There was no difference in thymic weight as a percentage of total body weight between CD1-E and Het-nude stocks at necropsy (Mann–Whitney test, $P = 0.08$). For panels C and D, groups 1 and 2 were combined; CD1-E, $n = 24$; Het-nude, $n = 26$. Student *t*-test, $P < 0.05$ was considered to be significant; error bars, SEM. NS, not significant.

Table 2. Body, thymic, and splenic weights for CD1-E and Het-nude Mice

Stock	*n*	Mean (SEM)	1 SD	95% CI	*P*
Body weight (g)[a, b]					
CD1-E	24	30.86 (1.3)	6.28	27.0–33.9	0.001
Het-nude	26	25.99 (0.5)	2.29	24.1–27.5	
Thymic weight (% of body weight)[a,b]					
CD1-E	24	0.24 (0.019)	0.095	0.20–0.27	NS, 0.08
Het-nude	26	0.27 (0.017)	0.085	0.24–0.33	
Splenic Wt (% of body weight)					
CD1-E	7	0.43 (0.03)	0.089	0.32–0.55	NS, 0.76
Het-nude	8	0.44 (0.03)	0.089	0.35–0.60	

[a]Groups 1 and 2 combined.
[b]Numbers include the mice that were negative controls (not exposed to soiled bedding or exposed to soiled bedding from an MNV–MHV-negative population).

seroconversion, would be needed to confirm an association between seroconversion and thymic morphology. Lymphocyte programming and seroconversion metrics are beyond the scope and overall goal of the current study.

Animal care programs are doing their part to use effective health monitoring programs that respect the principles of the 3Rs. Although some programs have been able to vastly reduce or eliminate the use of SBS animals, SBS may still be necessary for part of the health monitoring program. Facilities using SBS contribute to the 3Rs by using replacements to SBS whenever scientifically valid, using the minimal number of SBS animals necessary, and considering the use of strains or stocks that might otherwise be an excess part of a breeding program. The use of female Het-nude mice as SBS animals may provide a scientifically valid use in health-monitoring programs for animals that might otherwise be culled from athymic nude production colonies. In addition, Het-nude mice are 34% less expensive than CD1-E mice. This difference represents a cost saving exceeding $9,000 per year at our facility, where we purchase approximately 1200 sentinels annually.

Based on the results of this study, our facility is now using Het-nude mice as SBS animals in rodent barrier facilities. This decision was made in light of the Het-nude mice's adequate seroconversion to MNV and MHV, the fact that their use supports the 3Rs, and anecdotally that they were more docile for procedures, compared with CD1-E mice. A limitation of this study is its focus on MNV and MHV. Other institutions may

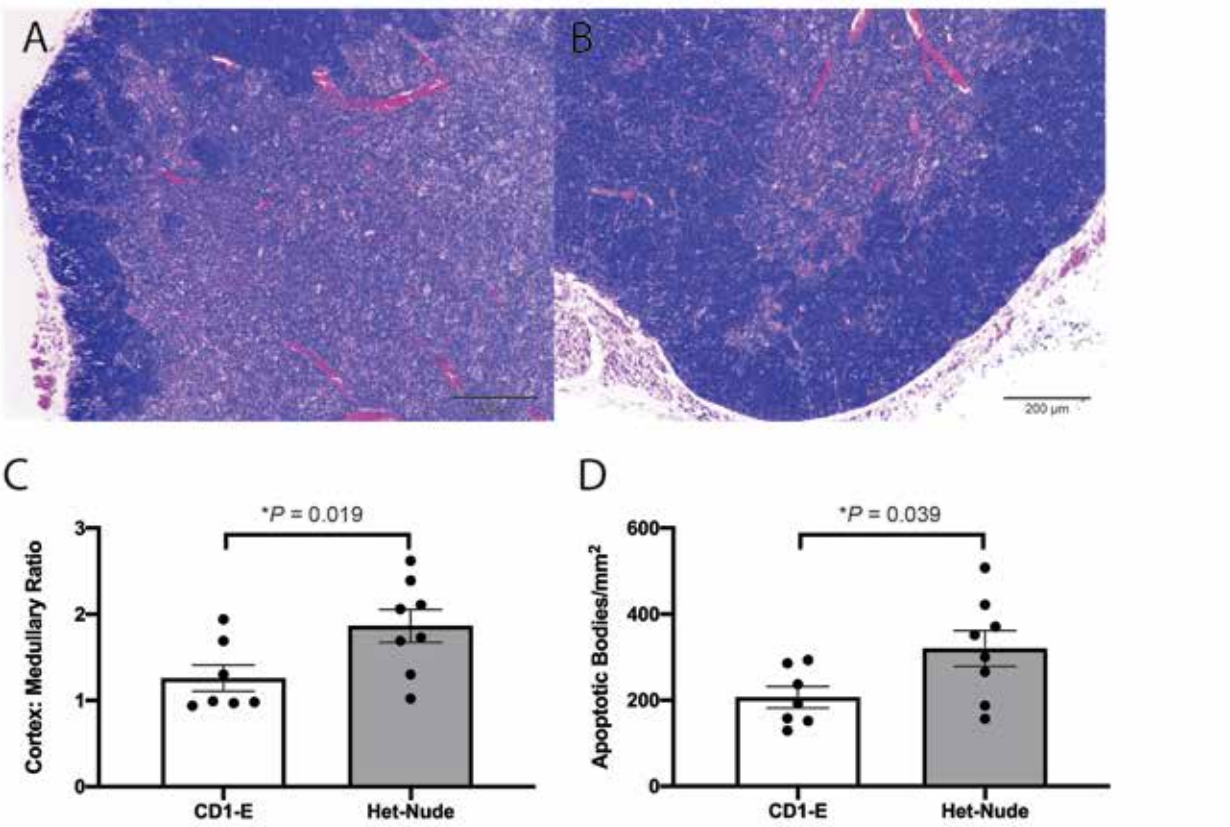

Figure 3. Histomorphometric analysis of thymus from CD1-E and Het-nude mice. Representative images of thymus from a (A) CD1-E and (B) Het-nude mouse. Hematoxylin and eosin stain; original magnification, 40×. (C) Het-nude mice had significantly increased cortex:medullary ratios due to larger cortices than CD1-E mice; Mann–Whitney test, $P = 0.019$. (D) Het-nude mice had significantly more cortical apoptotic bodies than CD1-E mice; Welch t test, $P = 0.039$. For panels C and D: CD1-E, n = 7; Het-nude, n = 8. $P < 0.05$ was considered to be significant; error bars, SEM.

choose to compare Het-nude and CD1-E mice to detect other viral, bacterial, or parasitic agents within rodent facilities before using Het-nude mice as SBS.

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