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Reliability of Soiled Bedding Transfer for Detection of Mouse Parvovirus and Mouse Hepatitis Virus

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Serologic monitoring of sentinel mice exposed to soiled bedding is a common method of detecting viral infections in mice. Because bedding transfer protocols vary, the sensitivity of this method has not been documented sufficiently. We examined the reliability of bedding transfer during various stages of infection with mouse parvovirus (MPV) and mouse hepatitis virus (MHV). Most mice exposed to bedding contaminated with MPV 0, 3, or 7 d previously seroconverted, whereas only mice exposed to bedding contaminated with MHV 4 h previously seroconverted, thus confirming the differing stabilities of these viruses. Index mice were inoculated with 30 times the infectious dose 50 (ID_{50}) of MPV or 300 ID_{50} of MHV. At 3 d, 1 wk, and 2 wk postinoculation (PI), we transferred 25, 50, or 100 ml of bedding to cages of sentinel mice. Viral infection and shedding by index mice was confirmed by serology and fecal polymerase chain reaction assay. Transfer of soiled bedding between mice in static cages induced seroconversion of sentinel mice most reliably during peak viral shedding (1 wk PI for MPV and 3 d PI for MHV). Soiled bedding transfer between mice in individually ventilated cages induced a higher prevalence of sentinel seroconversion to MPV and MHV than that after transfer between mice in static cages. Our findings indicate that although soiled bedding transfer is an effective method for detecting MHV and MPV under optimal conditions, the method is less than 100% reliable under many conditions in contemporary mouse facilities.

Abbreviations: ID_{50}, infectious dose 50; IVC, individually ventilated caging; MHV, mouse hepatitis virus; MPV, mouse parvovirus; PCR, polymerase chain reaction; PI, postinoculation; RT-PCR, reverse transcriptase–polymerase chain reaction

Microbiologic monitoring is vital to the health and use of laboratory mice. Critical decision-making about animal experimentation often relies on the quality and reliability of monitoring materials and methods. Therefore, accurate, sensitive, and affordable methods must be used for detecting infectious agents that can disrupt research involving mice. Most contemporary monitoring protocols use seroconversion of specific pathogen-free sentinel mice as the primary means to detect viral, bacterial, and parasitic infections. Scheduled transfer of soiled bedding from cages housing breeding or experimental mice to those housing sentinel mice is the primary means of eliciting antibody responses in sentinels.[1,9,10,17,19,33] However, protocols and standards for bedding transfer vary widely. Further, the accuracy and sensitivity of bedding transfer has not been assessed or optimized systematically for many infectious agents or in individually ventilated caging (IVC). The amount of bedding transferred and the frequency of transfer necessary to detect infection are likely to vary from agent to agent. This variability reflects the diverse properties of viruses, including their routes and duration of excretion, infectivity, and their stability in the environment, as well as host- and husbandry-related factors such as mouse age and immunologic status, caging type, cage-changing frequency, and colony turnover.[11,20,34] The present study assessed the reliability of soiled bedding transfer, using mouse parvovirus (MPV) and mouse hepatitis virus (MHV) as index agents because they are 2 of the most prevalent viruses in contemporary mouse colonies.[14,18] MPV is a nonenveloped DNA virus that causes persistent infection, is shed in feces, and is highly stable in the environment.[13,34] MHV is an enveloped RNA virus that causes acute infection, is shed in the feces, and is unstable in the environment.[2,11] We first sought to determine the ability of bedding containing quantified amounts of MPV or MHV to elicit seroconversion in sentinel mice. Our second aim was to determine the amount of soiled bedding from cages containing virus-infected mice necessary to transmit infection to sentinel mice at 3 time points after inoculation. Because the use of IVC is expanding rapidly, we also compared the effectiveness of bedding to elicit seroconversion of sentinel mice under both static and ventilated caging conditions.

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Materials and Methods

Mice. We obtained 4- to 6-wk-old, female, Swiss Webster mice (Tac:[SW]) from Taconic (Germantown, NY). Upon arrival at Yale, mice were seronegative for ectromelia virus, murine rotavirus, lymphocytic choriomeningitis virus, MHV, MPV, minute virus of mice, pneumonia virus of mice, reovirus, Sendai virus, and *Mycoplasma pulmonis* and were free of bacterial and parasitic infections. Mice were housed in a quarantine facility. Room conditions included a negative pressure differential relative to the corridor, a 12:12-h light cycle, and 10 to 15 air changes hourly. Mice were housed in IVC or nonventilated filter-top static isolator cages 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. Static cages were changed weekly, and IVC was changed biweekly in a class II biosafety cabinet within the animal room. 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.

Animal inoculations. Unanesthetized index mice were inoculated orally with 300 times the infectious dose 50 (ID_{50}) of MHV-Y (20 µl of a 10% weanling intestinal stock)[5] or oronasally with 30 ID_{50} of MPV-1d, formerly called wild-type mouse orphan parvovirus (20 µl of a 10% spleen stock).[28] Unanesthetized mice were inoculated oronasally with 20 µl of 10% mesenteric lymph node homogenates prepared from mesenteric lymph nodes of index mice 6 to 18 wk PI to determine whether infectious virus was present in lymph nodes.

Sample collection. A single fecal pellet was collected from the anus of each unanesthetized mouse while it was gently restrained; the pellet was frozen at –70 °C pending polymerase chain reaction (PCR) analysis. After euthanasia of mice by carbon dioxide overdose, mesenteric lymph nodes and small intestines were collected aseptically and frozen at –70 °C for PCR analysis. Blood was collected postmortem by cardiocentesis.

Serology. Sera were tested for MPV and MHV antibodies by use of immunofluorescent antibody assays as previously described.[30,31]

Nucleic acid assays. MPV DNA was isolated from feces, mesenteric lymph nodes, and small intestines by use of DNeasy Tissue kits (Qiagen, Valencia, CA) according to the manufacturer's instructions. PCR was performed with the DyNAmo SYBR Green qPCR kit (MJ Research, Waltham, MA) and primers specific for the MPV nonstructural gene.[7] The reaction conditions were: 2 min at 94 °C; 35 cycles of 30 s at 92 °C, 30 s at 50 °C, and 60 s at 72 °C; and 5 min at 72°C. MHV RNA was isolated from feces by use of RNeasy kits (Qiagen) according to the manufacturer's instructions. Reverse transcriptase–PCR (RT-PCR) was performed by use of Brilliant SYBR green qRT-PCR kits (Stratagene, La Jolla, CA) and primers specific for the MHV nucleocapsid gene.[6] The reaction conditions were: 30 min at 50 °C; 10 min at 95 °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. PCR primers were obtained from the WM Keck Foundation Biotechnology Resource Laboratory at Yale University. All PCR and RT-PCR assays included positive and negative controls.

Exposure of sentinels to 'virus-spiked' bedding. Fecal pellets were collected from a population of uninfected SW mice the day after their arrival at Yale, and aliquots of approximately 90 pellets (4 ml) were placed into 20 15-ml conical tubes. This volume was determined to be the average daily fecal output of 2 outbred female mice (data not shown). One random aliquot of 90 fecal pellets was tested by PCR and RT-PCR and was found to be free of MPV and MHV. Aliquots of fecal pellets (4 ml) were added to 120, 600, and 3000 ID_{50} of MHV and 6, 30, and 150 ID_{50} of MPV; virus was suspended in 10 ml of Dulbecco media containing 10% fetal bovine sera. Five minutes after addition of virus, each virus-soaked feces mixture was mixed into 400 ml of autoclaved corncob bedding in an autoclaved, static isolator cage. Two sentinel mice placed in each of 2 'virus-spiked' cages at 4 h, 3 d, and 7 d after addition of virus to bedding. The sentinels were tested for antibodies to MPV and MHV 22 d after their exposure to virus-spiked bedding.

Bedding transfer study in static isolator cages. Soiled bedding collected from mice, prior to their inoculation with virus, was used as 'uninfected soiled bedding.' On the day of inoculation, 5 mice were euthanized and sera were submitted for complete viral serology. All samples were determined to be free of viral antibodies. We inoculated 12 index mice with MPV and 12 with MHV. Index mice were housed 3 per cage in static isolator cages. Soiled bedding from these cages was transferred to 1 sentinel cage at 3 d, 1 wk, and 2 wk PI to represent the early, middle, and late stages of infection of index mice. We transferred 25, 50, or 100 ml of soiled bedding to static cages containing 400 ml of soiled bedding from uninfected mice, and 2 sentinel mice were added to each cage (Figure 1). Twenty-five ml of soiled bedding is the amount currently transferred from each cage in Yale's microbiologic monitoring protocol, and 400 ml is the average volume of soiled bedding added to each sentinel cage during each cage change cycle. At 3 d, 1 wk, and 2 wk PI, 25 ml of soiled bedding from each index cage and fecal samples from each index mouse were collected and stored at –70 °C until PCR analysis. In addition, at 1 wk PI, index mice from 1 cage per virus were placed into clean cages, and 2 mice were added to each of the soiled cages to serve as 'undiluted' bedding sentinels. Mice were tested for antibodies to MPV and MHV at 3 wk PI for index mice or 3 wk postexposure for soiled bedding sentinel mice.

This study was repeated with several minor changes. At 3 d, 1 wk, and 2 wk PI, a direct contact sentinel mouse was added to each index cage, and the number of bedding transfer sentinels was increased to 4 (2 cages of 2 mice) at each bedding volume and time point. MHV index mice were euthanized at 5 wk PI, and sera were tested for antibodies to MHV and MPV. To assess the duration of MPV shedding and transmission, feces were collected from index mice for PCR analysis at 4, 6, 10, 12, 14, 16, and 18 wk PI, and 2 contact sentinels were placed with 3 index mice at 4, 8, 12, and 16 wk PI. Mesenteric lymph nodes, feces, and small intestine were collected after euthanasia from MPV index and sentinel mice for PCR analysis. All sentinel mice were tested for antibodies to MPV and MHV 3 wk postexposure.

Bedding transfer study in IVC. Groups of 12 index mice were inoculated with either MPV or MHV and were housed 3 per cage per virus in IVC. Transfer and testing of bedding was performed as described for the second study in static caging, except that the sentinel mice were housed in IVC. At 3 d, 1 wk, and 2 wk PI, soiled bedding was added to 2 cages containing 2 sentinel mice and a direct contact sentinel mouse was added to each index cage. Contact sentinels were exposed to index mice for 1 wk. Index mice were euthanized at 5 wk PI, and blood and feces were collected for serology and PCR analysis. Bedding and contact sentinel mice were euthanized 3 wk postexposure, mesenteric lymph nodes were collected from MPV index and sentinel mice for PCR analysis, and sera were tested for antibodies to MHV and MPV.

The second IVC bedding study was performed using essentially the same methods as in the first IVC study, except that mesenteric lymph nodes were not collected for MPV PCR analysis.

Statistical analysis. Seroconversion rates were plotted as percentage of bedding transfer sentinel mice that seroconverted to each virus at each time point under 2 different housing conditions (static and IVC). For each virus, seroconversion rates were compared between time point and between housing types using a two-tailed Mann-Whitney U test.

Results

Exposure of mice to 'virus-spiked' bedding. Mice placed on bedding 4 h after the addition of virus-spiked feces showed dose-

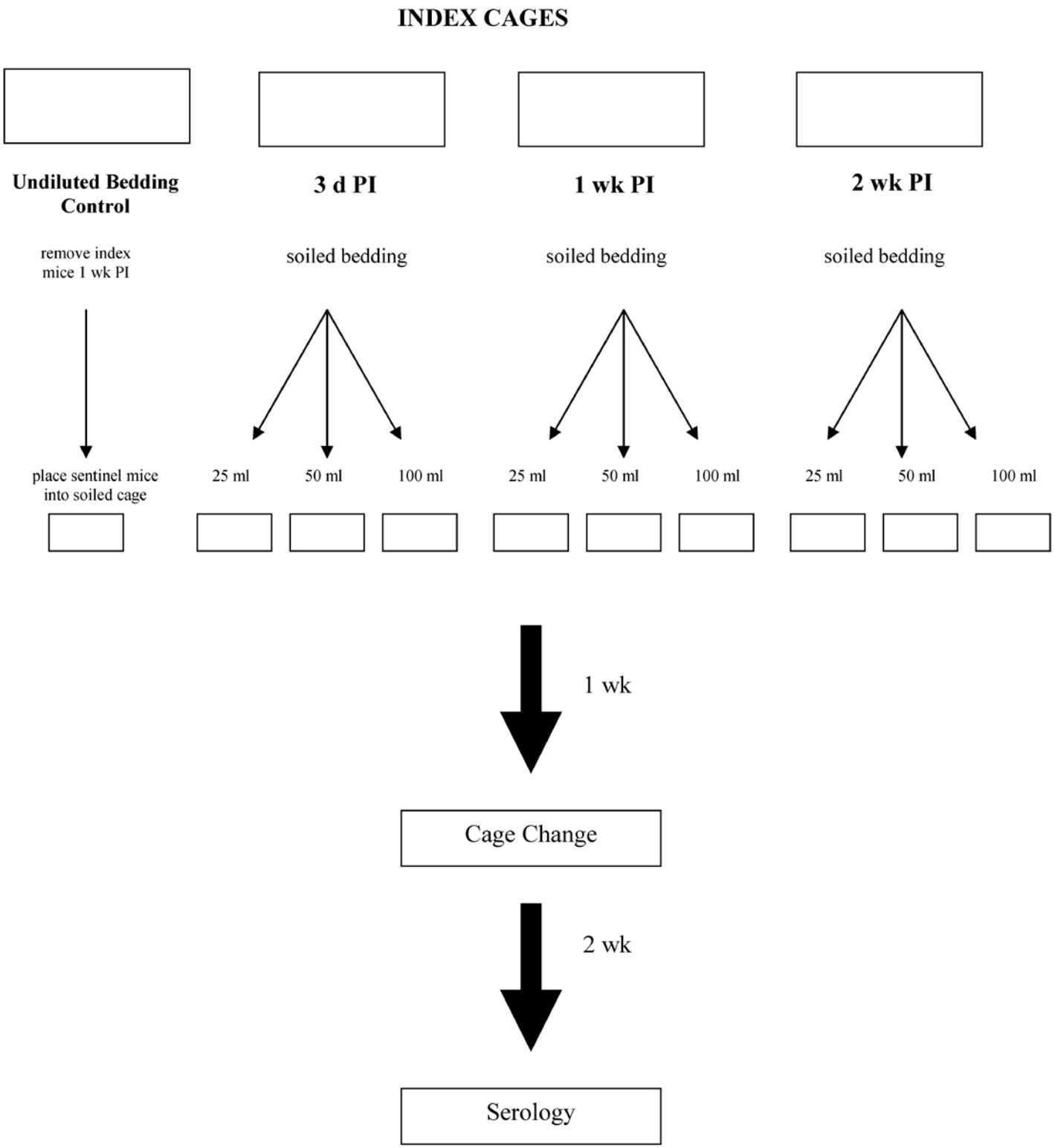

Figure 1. Schematic representation of soiled bedding transfer protocol. At the time of soiled bedding transfer, index mice were placed in clean cages.

dependent seroconversion (Table 1). Seroconversion occurred in all 4 mice after exposure to 150 ID_{50} of MPV but only in some of the mice at lower doses. In addition, 2 of 4 mice exposed to 3000 ID_{50} of MHV seroconverted, but none did so at lower doses. Exposure beginning on day 3 or 7 after MPV-spiked feces were added to bedding resulted in seroconversion among 16 of the 24 mice tested. In contrast, no mice placed on bedding containing MHV-spiked feces at these later time points seroconverted. These results are consistent with those obtained with rat parvovirus[34] and rat coronavirus[11] in that MPV was stable in the environment whereas MHV was relatively unstable.

Bedding transfer using static isolator cages. All 24 index mice

Table 1. Seroconversion rates in sentinel mice placed on bedding 'spiked' with mouse parvovirus (MPV) or mouse hepatitis virus (MHV)

Virus	No. of ID_{50} added to bedding	No. of seropositive sentinels among the 4 animals placed on the bedding at the indicated time after adding virus		
		4 h	3 d	7 d
MPV	6	1	0	2
	30	2	4	2
	150	4	4	4
MHV	120	0	0	0
	600	0	0	0
	3000	2	0	0

For each virus, bedding was spiked with 3 multiplicities of the respective ID_{50}, and sentinel mice (2 mice per cage; 4 sentinels exposed per data point) were placed on the bedding at 4 h, 3 d, or 7 d after addition of virus. Serology was performed 3 wk after exposure to virus-spiked bedding. Results are expressed as the number of seropositive sentinels.

in both experiments seroconverted only to the inoculated virus. Fecal shedding of MPV was detected in more than 90% of index mice at all time points (23 of 24 mice on day 3 PI, 23 of 23 at 1 wk PI, and 22 of 24 at 2 wk PI). Similarly, pools of 10 fecal pellets removed from the soiled bedding collected from MPV index cages 3 d, 1 wk, and 2 wk PI all were (4 of 4) positive for MPV by PCR. Fecal shedding of MHV was detected in at least 85% of index mice 3 d (23 of 24 mice) and 1 wk (21 of 24 mice) PI and in 50% (12 of 24) of index mice 2 wk PI. Pools of 10 fecal pellets removed from the soiled bedding collected from MHV index cages 3 d and 1 wk PI all were (4 of 4 for each time point) positive for MHV by RT-PCR but negative at 2 wk PI.

Seroconversion to MPV was elicited only in sentinels exposed to the highest dose (100 ml) of bedding 3 d PI, and only among half of these mice (Figure 2 A). However, more than two thirds of the sentinels seroconverted after exposure to soiled bedding 1 wk PI, regardless of the volume (25, 50, or 100 ml) of bedding transferred (Figure 2 A). In addition, all 4 sentinels placed in an index cage after removing the index mice 1 wk PI seroconverted to MPV. Exposure to soiled bedding 2 wk PI resulted in seroconversion of 50% or fewer sentinels (Figure 2 A). Sentinels placed in contact with MPV index mice for 1 wk at 3 d or 1 wk PI, but not at 2 wk PI, seroconverted

Seroconversion to MHV was elicited in all sentinel mice exposed to soiled bedding 3 d PI regardless of the volume of bedding transferred (Figure 2 B). One third (2 of 6) of the sentinel mice exposed to 25, 50, or 100 ml of soiled bedding from cages housing MHV-infected mice 1 wk PI seroconverted (Figure 2 B), whereas all 4 sentinels exposed to 400 ml of soiled bedding seroconverted. One third of mice exposed to either 50 ml or 100 ml of soiled bedding from MHV index cages 2 wk PI also seroconverted, whereas those exposed to only 25 ml did not (Figure 2 B). All sentinels placed in contact with MHV index mice at 3 d, 1 wk, or 2 wk PI seroconverted. Under static conditions, seroconversion to MPV was significantly higher ($P < 0.02$) at 1 wk PI as compared with values at 3 d or 2 wk PI (Figure 3 A), and seroconversion to MHV was significantly higher ($P < 0.005$) at 3 d as compared to values at 1 or 2 wk PI (Figure 3 B).

Bedding transfer using IVC. All index mice seroconverted only to the virus with which they were inoculated. Fecal shedding

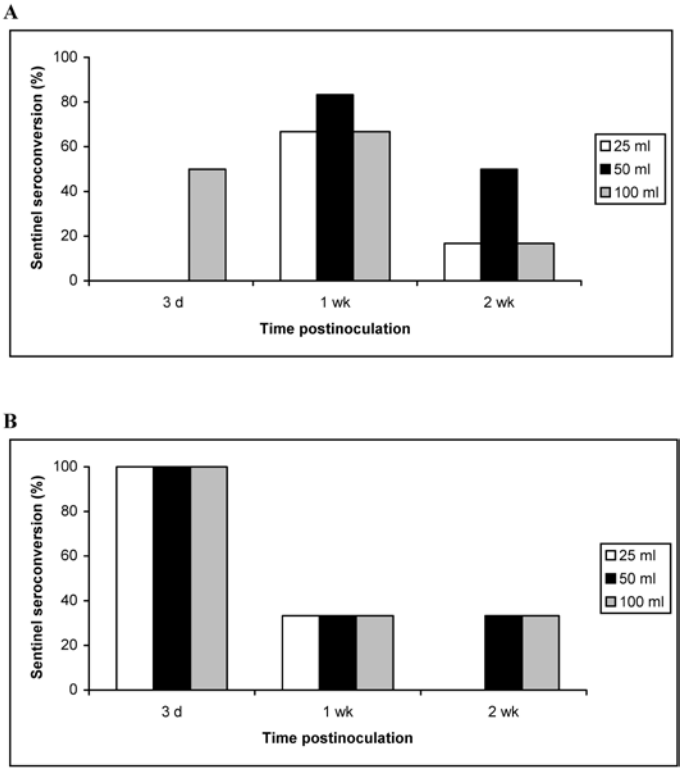

Figure 2. Seroconversion rates in sentinel mice housed in static cages and exposed to soiled bedding from mice inoculated with (A) MPV or (B) MHV. Data are expressed as seroconversion rate (%) for each bedding volume at each time point and represent the combined results of duplicate trials (n = 6 sentinel mice per dose per time point).

of MPV was detected by PCR in more than 80% of index mice through 2 wk PI (24 of 24 mice at 3 d PI, 24 of 24 at 1 wk PI, and 20 of 24 at 2 wk PI). Pools of 10 fecal pellets removed from the soiled bedding collected from MPV index cages 3 d, 1 wk, and 2 wk PI were all (4 of 4) positive for MPV by PCR. Fecal shedding of MHV was detected by RT-PCR in all 24 index mice at 3 d, 1 wk, and 2 wk PI, and pools of 10 fecal pellets from soiled bedding collected on these days were also all (4 of 4) positive for MHV by RT-PCR.

Under IVC conditions, seroconversion to MPV was greatest at 1 wk, although this difference was not statistically significant (Figure 3 A). A high percentage of mice exposed to soiled bedding from cages housing MPV mice 3 d PI seroconverted regardless of bedding dose (Figure 4 A). Further, all sentinels exposed to soiled bedding from MPV index mice 1 wk PI seroconverted regardless of bedding dose (Figure 4 A). For bedding transfers 2 wk PI, sentinels seroconverted at all bedding doses, but the overall prevalence had decreased (50% to 75%; Figure 4 A). Most sentinels placed in contact with MPV index mice seroconverted (2 of 2 mice at 3 d PI, 3 of 4 at 1 wk PI, and 1 of 2 at 2 wk PI). All sentinel mice exposed to soiled bedding from cages housing MHV index mice seroconverted to MHV at all time points and bedding doses (Figure 4 B). All sentinels placed in contact with MHV index mice at 3 d, 1 wk, or 2 wk PI seroconverted. Overall seroconversion rates were greater under IVC compared with static housing conditions. This difference was significant ($P < 0.02$) at 3 d and 2 wk PI for MPV and at 1 wk and 2 wk PI for MHV (Figure 3 A, B).

Duration of MPV infection. Fecal shedding of MPV was not detected at time points beyond 4 wk PI, and contact sentinels

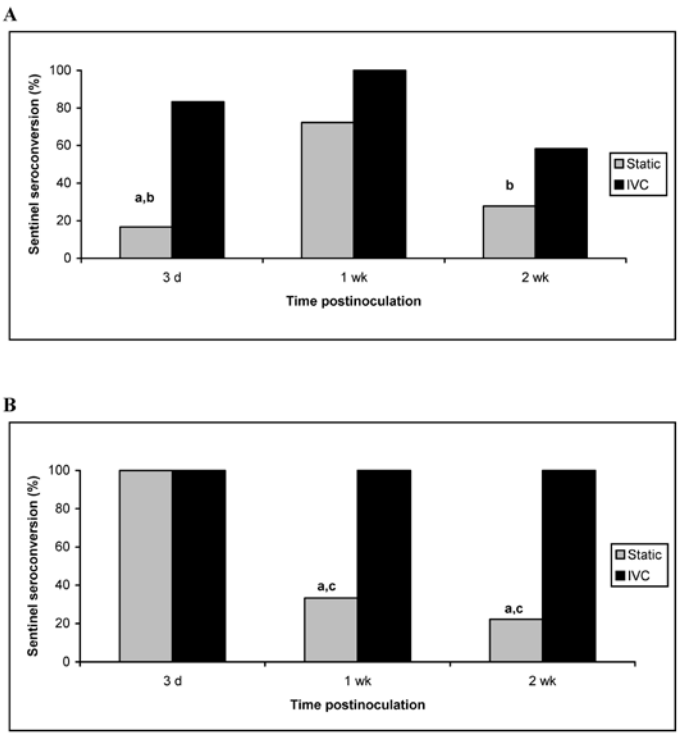

Figure 3. Seroconversion rates in sentinel mice housed in static cages compared with individually ventilated cages and exposed to soiled bedding from mice inoculated with (A) MPV or (B) MHV. Data are expressed as seroconversion rate (%) at each time point and represent the combined results of all mice at each time irrespective of amount of bedding transferred from index cages (n = 18 sentinel mice in static cages at each time point and n = 24 sentinel mice in IVC at each time point). a, $P < 0.02$ in 2-tailed Mann-Whitney *U* test as compared with value for IVC at the same time point; b, $P < 0.02$ in 2-tailed Mann-Whitney *U* test as compared with value for static cages at 1 wk PI; c, $P < 0.005$ in 2-tailed Mann-Whitney *U* test as compared with value for static cages at 3 d PI.

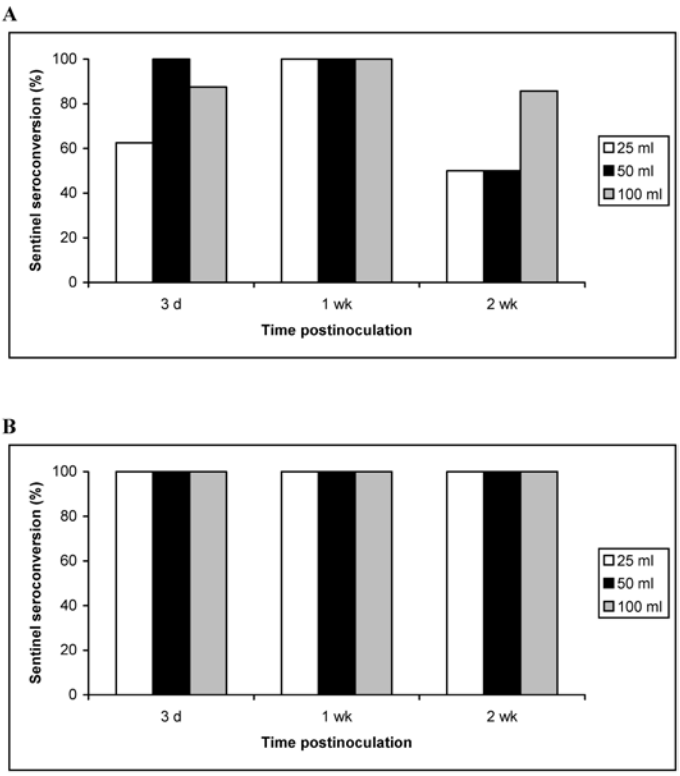

Figure 4. Seroconversion rates in sentinel mice housed in individually ventilated cages, and exposed to soiled bedding from mice inoculated with (A) MPV or (B) MHV. Data are expressed as seroconversion rate (%) for each bedding volume at each time point and represent the combined results of duplicate trials (n = 8 sentinel mice per dose per time point).

cohoused with index mice at 4 and 6 wk PI did not seroconvert. However, mesenteric lymph nodes from all 3 index mice tested at 6, 10, 14, and 18 wk PI contained MPV DNA. Mesenteric lymph node homogenates from 4 and 6 wk PI, but not later time points, were presumed to contain infectious virus, because mice inoculated oronasally with these homogenates seroconverted to MPV. All samples of feces and small intestine from these mice were negative for MPV DNA after 4 wk.

Discussion

These results confirm that MPV is stable in the environment for at least 7 d. Therefore, bedding from cages housing MPV-infected mice may contain infectious bedding well beyond the period of viral shedding, thus providing a wide window of opportunity to detect infection by use of bedding transfer. Similar ease of detection would be expected with other environmentally stable, nonenveloped viruses that cause intestinal infections and are shed in the feces (for example, murine rotavirus, murine norovirus, and Theiler murine encephalomyelitis virus). Prior studies have reported that the duration of MPV transmission from adult immunocompetent mice is 2 to 4 wk.[28,31] Our results were similar in that fecal shedding and MPV transmission to contact sentinels ceased between 2 and 4 wk PI. Nevertheless, MPV DNA was detected in mesenteric lymph nodes through 18 wk and infectious virus remained in the mesenteric lymph nodes for at least 6 wk. These results confirm a recent report that MPV DNA was detectable by PCR of mesenteric lymph nodes from endemically infected Sencar mice between the ages of 6 to 35 wk, whereas MPV DNA was not detectable in feces collected from mice older than 17 wk of age.[3] Therefore, mice harbored infectious MPV in their mesenteric lymph nodes between 4 and 6 wk PI, but MPV infection was not detected in sentinel mice or by fecal PCR. This finding raises the possibility that reactivation of viral shedding could occur under conducive conditions, such as immunosuppression. Future studies are planned to address this question.

By contrast, MHV-inoculated bedding did not elicit seroconversion in sentinels exposed after 4 h. This result suggests that bedding transfers from cages housing MHV-infected mice will be effective only during the period of active shedding. Although MHV generally causes acute infection, the period of active shedding varies between mouse strains. For example, C57BL/6 mice can transmit virus for 2 wk, BALB/c mice can transmit virus for 4 wk, and some strains of immunocompromised mice can transmit MHV for several months.[5,12,25,27] Therefore the period of time during which MHV can be detected by use of bedding transfer is likely to be mouse strain-dependent.

MPV and MHV were transmitted to sentinels via soiled bedding, but the reliability of the method was dependent on the timing of bedding transfer. In static cages, MPV seroconversion was significantly higher at 1 wk PI as compared with 3 d or 2 wk PI (Figure 3 A) and MHV seroconversion was significantly higher at 3 d PI as compared to 1 or 2 wk PI (Figure 3 B). These results

correspond with the peak of MPV infection in the intestine (5 to 10 d PI)[31] and MHV infection in the intestine (2 to 5 d PI).[2] It is important to note that in 17 of 48 cages housing sentinels on MPV-containing bedding in which seroconversion occurred, only 1 of the 2 mice seroconverted. This finding suggests that the virus content of soiled bedding was frequently close to the ID_{50} and that transmission between sentinels was inefficient. This hypothesis of inefficient transmission of MPV was supported by the finding that only 75% of sentinels placed in contact with MPV index mice between 3 d and 2 wk PI seroconverted. In contrast, when MHV seroconversion occurred, both sentinels per cage always were seropositive, suggesting that efficient transmission of MHV occurred between sentinels.

Seroconversion rates among soiled bedding sentinels housed in IVC were significantly higher for MPV soiled bedding sentinels at 3 d PI and for MHV soiled bedding sentinels at 1 and 2 wk PI than were rates in soiled bedding sentinels housed in static cages (Figure 3 A, B). These results were somewhat surprising, because we anticipated that enhanced desiccation of feces in IVC would accelerate viral inactivation. This counterintuitive result may indicate that higher ammonia concentrations in static cages increase inactivation of virus.[8,16,21,26,32] We used corncob bedding, which has been reported to result in lower ammonia concentrations than do wood, paper, and cellulose bedding, and therefore viral inactivation potentially could be greater with other bedding types.[23] Another possible explanation for this result is that increased airflow in IVC may have dispersed virions more efficiently throughout the cage, resulting in increased exposure of the sentinel mice to virus.[15]

Effective exposure of sentinels to soiled bedding is affected by the volume of bedding transferred, fecal load per unit volume of bedding, virus concentration in feces, and the frequency of transfer. These factors, in turn, are influenced by the percentage of soiled bedding that contains virus and thus is influenced by the proportion of cages sampled that contain mice which are shedding or recently have been shedding virus and whether all of the mice in a cage are shedding virus at the time of bedding transfer. This presents a problem for serologic monitoring for MPV, because MPV often results in small pockets of infection within colonies of mice housed in static isolator cages or IVC and is not transmitted well between mice within a cage. Therefore the virus content of soiled bedding pooled from several cages, used for exposure of sentinels, often will be low. The viral concentration of soiled bedding also is affected by amount of fecal material that has accumulated in the cage and thus the rate of cage changing and the number of mice housed per cage. In our studies, all index cages housed 3 mice, but because static cages were changed at 1 wk PI, sentinels exposed to soiled bedding from static cages at 2 wk PI probably were exposed to less fecal material from infected mice than those exposed to soiled bedding from IVC. For highest efficiency of viral transfer, the sample of soiled bedding removed must be representative of all of the bedding in the cage or even biased by selecting bedding from areas containing the highest density of feces. Human nature sometimes leads to sampling of the cleanest, driest bedding in the cage, introducing sample bias into the process and resulting in failure to detect infection in a colony.

The fecal concentration of virus also can be influenced by age and strain of mouse. Viral concentration in feces is highest during the peak of infection and is often higher in genetically altered and immunodeficient mice, which cannot control viral replication, than in outbred mice.[5,6] For example, ICR mice are more susceptible to MPV at 4 or 8 wk of age than at 12 wk, and 12-wk-old DBA/2, C3H/HeN, and BALB/c mice are more susceptible to MPV than are 12-wk-old C57BL/6 mice.[4] Our studies used 4- to 6-wk-old female outbred Swiss Webster mice as sentinels, because they are the mice used in Yale's quality assurance monitoring program and because 4- to 6-wk-old outbred mice would be expected to be more susceptible to infection than would older outbred mice or inbred mice. These factors indicate that the choice of sentinel age and strain may affect serologic detection. In addition, the use of inbred mice as sentinels could adversely affect the sensitivity of serologic monitoring. Many quality assurance monitoring programs, including the program used at Yale University, use repeated transfer of soiled bedding (for example, biweekly over a 6-mo period).[17,22] During the later part of the monitoring program, when susceptibility to MPV infection in the sentinels decreases, MPV infection could be missed.

In summary, we found that transfer of soiled bedding is an effective method to detect MHV and MPV infection under optimal conditions, but this method is less reliable under conditions common to contemporary mouse colonies. This method detected MPV and MHV at all time points under both static and ventilated conditions, but in most cases (with the exception of MHV in ventilated cages), the rate of seroconversion in sentinel mice was dependent on the volume of soiled bedding sampled and the stage of infection at which it was transferred. Bedding transfer also is likely to be less effective for viruses transmitted only by contact or aerosol.[1,7,9,10] Therefore, a multifaceted quality assurance program is recommended and should include soiled bedding sentinels as well as other methods, such as contact sentinels, direct monitoring of a subset of colony mice, and environmental monitoring. Frequent sampling and the use of a sufficiently large sample size to detect infections present at low prevalence also will increase the likelihood of detecting an infection early.[7,24,29]

Acknowledgments

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