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Efficacy of Three Microbiological Monitoring Methods in a Ventilated Cage Rack

Susan R. Compton, PhD,[1,*] Felix R. Homberger, DVM, PhD,[2] Frank X. Paturzo,[1] and Judy MacArthur Clark, DVMS[3]

The use of individually ventilated caging (IVC) to house mice presents new challenges for effective microbiological monitoring. Methods that exploit the characteristics of IVC have been developed, but to the authors' knowledge, their efficacy has not been systematically investigated. Air exhausted from the IVC rack can be monitored, using sentinels housed in cages that receive rack exhaust air as their supply air, or using filters placed on the exhaust air port. To aid laboratory animal personnel in making informed decisions about effective methods for microbiological monitoring of mice in IVC, the efficacy of air monitoring methods was compared with that of contact and soiled bedding sentinel monitoring. Mice were infected with mouse hepatitis virus (MHV), mouse parvovirus (MPV), murine rotavirus (agent of epizootic diarrhea of mice [EDIM]), Sendai virus (SV), or *Helicobacter* spp. All agents were detected using contact sentinels. Mouse hepatitis virus was effectively detected in air and soiled bedding sentinels, and SV was detected in air sentinels only. Mouse parvovirus and *Helicobacter* spp. were transmitted in soiled bedding, but the efficacy of transfer was dependent on the frequency and dilution of soiled bedding transferred. Results were similar when the IVC rack was operated under positive or negative air pressure. Filters were more effective at detecting MHV and SV than they were at detecting MPV. Exposure of sentinels or filters to exhaust air was effective at detecting several infectious agents, and use of these methods could increase the efficacy of microbiological monitoring programs, especially if used with soiled bedding sentinels. In contemporary mouse colonies, a multi-faceted approach to microbiological monitoring is recommended.

The use of individually ventilated caging (IVC) to house rodents is increasing rapidly and presents new challenges for effective microbiological monitoring. Since each cage is, theoretically at least, its own biocontainment zone, traditional methods such as exposure of sentinels to airborne infectious agents present in the room are inappropriate. Exposure of sentinels to soiled bedding is the most common method used to monitor the microbiological status of rodents housed in IVC, but this method is labor and time intensive, introduces a potential hazard into the cage-change station environment (a box with potentially infective bedding), and is not effective at detecting infectious agents such as Sendai virus and cilia-associated respiratory bacillus, which are not transmitted by the fecal-oral route (1, 7, 8). Methods that exploit the characteristics of IVC have been developed, but to our knowledge, their efficacy has not been systematically investigated. Air exhaust from the IVC rack can be monitored by testing of sentinels housed in a specially designed cage that receives a portion of the exhaust air from the rack as its supply air source, or by testing of gauze filters placed on the inner surface of the exhaust air port of the IVC rack.

In an effort to aid laboratory animal personnel in making informed decisions about the most sensitive, specific, and economical methods for microbiological monitoring of rodents housed in IVC, the efficacy of these two air monitoring methods was compared to each other, to traditional placement of soiled bedding in sentinel cages, and to the placement of contact sentinels in cages housing experimentally infected mice. Mice were inoculated experimentally with five of the most common murine infectious agents (mouse hepatitis virus [MHV], mouse parvovirus [MPV], murine rotavirus [agent of epizootic diarrhea of mice {EDIM}], Sendai virus [SV] and *Helicobacter* spp. [11]). These agents vary in their infectivity, duration of infection, environmental stability, and size. Mouse hepatitis virus is an 80- to 160-nm enveloped single-stranded RNA virus that is environmentally labile (the closely related rat coronavirus is stable for 2 days at room temperature), is highly contagious, and causes acute intestinal infection (5). Mouse parvovirus is an 18- to 25-nm DNA virus that is environmentally stable (rat virus, a closely related parvovirus, is stable at room temperature for at least 35 days), is moderately contagious, and causes persistent systemic infection, with virus shedding in the urine, feces, and expired air (10). Murine rotavirus (EDIM) is a 70- to 75-nm double-shelled, segmented, double-stranded RNA virus that is environmentally labile (stable for several days), is highly contagious, and causes acute intestinal infection (21). Sendai virus is a 150- to 250-nm, enveloped, single-stranded RNA virus that is environmentally labile, is highly contagious, and causes acute infection of the lungs and is transmitted by the respiratory-oral route (14). *Helicobacter* spp. are gram-negative 5- to 10-µm spiral bacteria that are environmentally labile, are highly contagious, and cause persistent infections of the gastrointestinal tract and liver (25).

Given the speed of polymerase chain reaction (PCR)-based assays to detect an infectious agent's nucleic acids, the testing of gauze filters that have been exposed to the exhaust air from the

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[1]Section of Comparative Medicine, Yale University School of Medicine, New Haven, Connecticut; [2] Research Animal Resources Center, Weill Medical College of Cornell University and the Memorial Sloan-Kettering Cancer Center, New York, New York; and [3]BioZone Inc., Fort Mill, South Carolina.
[*]Corresponding author.

IVC could be useful in an infectious disease outbreak, to rapidly determine the distribution of an infectious agent in the laboratory animal colony, and to confirm when an agent has been successfully eradicated. The lability of nucleic acids and the turnover of infectious agents on gauze filters have been postulated, but have never been formally tested, as possible drawbacks of using PCR-based detection of infectious agents on gauze filters as a routine monitoring method. The lability of the five infectious agents on gauze filters placed on the inner surface of the exhaust air port of the IVC rack was tested. The results of the study reported here should aid laboratory animal personnel in making informed decisions on applicable methods to use in routine microbiological monitoring of rodents housed in IVC. Because exhaust air monitoring methods are less labor-intensive, and therefore are more cost-efficient than is the traditional soiled bedding monitoring, the use of air monitoring alone, or in combination with soiled bedding monitoring, for infectious agents could reduce animal use charges.

Materials and Methods

Mice. Murine pathogen-free (MPF), 4- to 6-week-old female Swiss Webster mice (Tac:[SW]) were obtained from Taconic (Germantown, N.Y.). Mice were seronegative for ectromelia virus, mouse rotavirus (EDIM), lymphocytic choriomeningitis virus, mouse hepatitis virus (MHV), mouse minute virus, mouse parvovirus (MPV), pneumonia virus of mice, reovirus, SV, *Mycoplasma pulmonis*, and on arrival, were free of bacterial and parasitic infections. All animal procedures were approved by the Yale Animal Care and Use Committee, and animal care was in accordance with the ILAR *Guide for the Care and Use of Laboratory Animals*.

Mice were housed, four to a cage, in type-22 cages (440-cm^2 floor area) in a quarantine facility, and room conditions included a negative pressure differential relative to the corridor, a 12:12-h light cycle, and 10 to 15 air changes/h. Mice were housed in a single-sided, 81-cage IVC rack (BioZone Inc. Fort Mill, S.C.) equipped with a DigiFlow System and two BioScreen cages, or in static cages (Polysulfone Standard mouse cage, ACE, Allentown, Pa.) equipped with filter cage tops. Mice were housed on sterilized corncob bedding and were fed sterilized standard rodent chow (5010 PMI, Purina Mills, St. Louis, Mo.) and hyperchlorinated water ad libitum by use of a water bottle. Static cages with filter cage tops were changed every 7 days within a class-II biosafety cabinet in the animal room. The IVC cages, but not lids, were changed every 14 days within a class-II biosafety cabinet in the animal room. Animal care personnel were "blinded" as to which cages contained experimentally infected mice (Fig. 1).

Infectious agents. Index mice to be experimentally infected with MHV were inoculated orally with 3×10^3 median mouse infective doses of MHV-Y (20 µl of 10% weanling intestinal stocks). Index mice to be experimentally infected with SV were inoculated intranasally with 10^5 median tissue culture infective doses ($TCID_{50}$) of SV (20 µl of 10% lung stocks). Index mice to be experimentally infected with EDIM were inoculated orally with 10 median mouse infective doses of murine rotavirus (20 µl of 10% infant intestinal stocks). Index mice to be experimentally infected with MPV were inoculated oronasally with 10^5 $TCID_{50}$ of MPV-1a (20 µl of an L3 cell stock) or 30 $TCID_{50}$ of MPV-1d, formerly called wild-type mouse orphan parvovirus, (20 µl of a 10% spleen stock [15]).

A

M			Pa		U	Hm		
U		U		S	R	U		M
	U	Hm					Pa	
S			R	U		M		
U	Pa						U	Hm
	S	U	M	Hm			R	
R		U	U	U	S	Pa		
am	D1							
As	A1	D2	D3	D4	D5	D6	B	B

B

M*	U	U	Pa		Pd	Hh	U	
U		U		S*	R	U		M
	Pd*	Hh	U		U	U	Pa*	
S			R*	U		M*	U	U
U	Pa*						Pd	Hh*
	S	U	M	Hh*			R*	U
R		U	Pd*		S*	Pa		
am	D1							
As	Al	D2	D3	D4	D5	D6	B	B

Figure 1. (A) Individual ventilated cage (IVC) rack configuration for positive air pressure experiment. (B) The IVC rack configuration for negative air pressure experiment.

Al = long-term exhaust air sentinels
As = short-term exhaust air sentinels
D1–D6 = timed soiled bedding positive controls
B = soiled bedding sentinels
Hh = *Helicobacter hepaticus*
Hm = *Helicobacter muridarum*
M = MHV (mouse hepatitis virus)
Pa = MPV-1a (mouse parvovirus)
Pd = MPV-1d
R = murine rotavirus (EDIM)
S = Sendai virus
U = uninoculated
am = airflow monitoring
*Cage-top filters

The BALB/c T-cell receptor alpha knockout index mice (BALB/c-*Tcra*^{tm1mom}) naturally infected with *H. muridarum* were culled from the Yale University animal colonies. Prior to initiation of the study, Swiss Webster index mice were infected by contact for

Table 1. Primers for polymerase chain reaction (PCR) assays

Organism	Forward primer position : sequence	Reverse primer position : sequence
Rotavirus[a]	8 : AAAGAGAGAATTTCCGTTTG	939 : GTAGAACACTTGCCATT
Helicobacter sp.	380 : CGTGGAGGATGAAGGTTTTAG	1372 : CCGACTTAAGGCGAATACAAC
MHV	512 : GTCATGAGGCTATTCCTACTA	1027 : ATACACATCTTTGGTGGG
MPV	1059 : CACTGCGCAGGAAACTAAG	1812 : CAAAGTCACCAGGCAATGTA
SV	399 : GGAGTAAACGCCGATGTCAAA	1274 : CCCTTGGCTGTATCCGTCACT

[a]Agent of epizootic diarrhea of mice (EDIM).
MHV = mouse hepatitis virus; MPV = mouse parvovirus; SV= Sendai virus.

2-4 weeks with a second group of BALB/c T-cell receptor alpha knockout mice that were naturally infected with *H. hepaticus*.

Assays for infectious agents. Blood was collected from the retro-orbital sinus of mice under methoxyfluorane-induced anesthesia or by cardiocentesis following euthanasia by inhalation of carbon dioxide. Sera were tested for antibodies specific for MHV, MPV, murine rotavirus, and SV using immunofluorescent antibody assays as previously described (3, 16-18). Fecal specimens were collected directly from the anus of individual mice, and DNA was isolated from feces or gauze filters (NuGauze rayon/polyester formed fabric sponges: Johnson and Johnson, Arlington, Tex.) using DNeasy Tissue kits (Qiagen, Valencia, Calif.) and following the manufacturer's instructions. A PCR assay was performed using *Taq* polymerase (Roche Molecular Biochemicals, Indianapolis Ind.) and primers specific for the MPV nonstructural gene or the *Helicobacter* species 16s rRNA gene (Table 1). The reaction cycles for the PCR assay were: 2 min at 94°C; 35 cycles of 30 sec at 92°C, 30 sec at 50°C, 60 sec at 72°C; and 5 min at 72°C. The PCR products were electrophoresed on 1% agarose gels, stained with ethidium bromide, and visualized by use of UV illumination.

Reverse transcriptase-PCR (RT-PCR) assays were performed on RNA isolated from feces, gauze filters, or calcium alginate swabs (Fisher Scientific, Houston, Tex.) using RNeasy kits (Qiagen) and following the manufacturer's instructions. The RT-PCR assay was performed using the Superscript One-Step RT-PCR System (Invitrogen, Carlsbad, Calif.) and primers specific for the murine rotavirus VP7 outer capsid glycoprotein gene, the MHV nucleocapsid gene, or the SV nucleoprotein gene (Table 1). The reaction cycles used for RT-PCR assay were: 30 min at 50°C; 2 min at 94°C; 40 cycles of 15 sec at 94°C, 30 sec at 50°C, 90 sec at 58°C; and 10 min at 72°C. The RT-PCR products were electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized by use of UV illumination. The PCR primers were obtained from the W. M. Keck Foundation Biotechnology Resource Laboratory at Yale University. All RT-PCR and PCR assays included positive and negative controls. Sterile pieces of NuGauze (approx. 1 × 1 cm) and sterile calcium alginate swabs were used as sampling materials because previous studies in our laboratory have indicated that MHV RNA, MPV DNA, and *H. hepaticus* DNA can be efficiently isolated, in the absence of PCR inhibitors, from these materials (4, 6).

Design of positive air pressure experiment. The IVC rack was configured to house 20 cages of experimentally infected index mice, 24 cages of uninoculated mice, 10 cages of sentinel mice, and 27 empty cages (Fig. 1A). The 20 cages of experimentally infected mice were systematically located throughout the IVC rack in such a way that each two rows of cages contained at least one cage of index mice infected with each agent (Fig. 1A, positions indicated in red, blue, green, yellow, and purple). The IVC rack was commissioned with an airflow of 120 air changes/h and a cage differential pressure of +1.5 ± 0.5 Pascals (Pa). These values were confirmed to be uniform between all cages on the IVC rack. This airflow and differential pressure was maintained in the IVC rack throughout the experiment. The seemingly high air changes per hour used in this experiment were possible because the design of IVC rack used, with large cage top supply and exhaust air openings, results in a gentle low-pressure airflow through each cage even at high air-change rates (13). This low-pressure airflow prevents the noise and low humidity-related health problems sometimes encountered in IVC racks with small cage openings when they are operated at over 70 air changes/h (23).

Index mice/contact sentinels. Twelve mice each were infected with one of the following viruses—MHV, MPV, murine rotavirus, and SV; eight mice were infected with *H. muridarum* (Fig. 1A; M, Pa, R, S, and Hm cages). These mice served as the index animals from which shedding of infectious agents was monitored. One contact sentinel mouse was placed in each cage of virus-inoculated mice, and two contact sentinel mice were placed in each cage of *H. muridarum*-infected mice at postinoculation day (PID) 2 (group C). Contact sentinels remained in contact with index mice for the duration of the experiment. Infection of index mice was confirmed by results of viral serologic testing, of *Helicobacter* and MPV PCR analysis of DNA extracted from feces, of MHV and murine rotavirus RT-PCR analysis of RNA extracted from feces, and of SV RT-PCR analysis of RNA extracted from cage swab specimens at postinoculation week (PIW) 2, 4, 6, 8, and 12.

Soiled bedding sentinels (group B). Eight mice housed in 2 cages (Fig. 1A; B cages) received pooled soiled bedding from 12 to 13 cages, including at least 1 cage of MHV-, MPV-, murine rotavirus-, SV-, *H. muridarum*-infected index mice, at PIW 2, 4, 6, 8, and 10. The pool of soiled bedding was generated by mixing 2 ounces of bedding from each cage in two rows at each time point (week 2: rows 1 and 2, week 4: rows 3 and 4, week 6: rows 5 and 6, week 8: rows 7 and 1, and week 10: rows 2 and 3). Infection of sentinel mice was detected by use of viral serologic testing and *Helicobacter* PCR analysis of fecal specimens at PIW 4, 6, 8, and 12.

Exhaust air sentinels (group A). Mice housed in two BioScreen cages were used to monitor which infectious agents were transmitted to sentinel mice by exhaust air. Each BioScreen cage received a portion of the exhaust air from the IVC rack prior to HEPA filtration. The BioScreen cages were operated under positive pressure relative to the room, and were ventilated at 120 air changes/h, similar to the ventilation for the other IVC cages. Four mice in one BioScreen cage (Fig. 1A, Al cage) were used as long-term exhaust air sentinels and were monitored throughout the 12-week experiment. Mice in the second BioScreen cage (Fig. 1A, As cage) were used as short-term exhaust air sentinels to determine the time frame during which each agent was transmissible in exhaust air. Four mice were

placed in the second cage at the initiation of the experiment, and were moved to a static cage equipped with filter cage top at PIW 2. Additional sets of 4 mice monitored the exhaust air passing through the short-term exhaust air cage from PIW 2 through 4, 4 through 6, 6 through 8, 8 through 10, and 10 through 12. Short-term exhaust air sentinels were evaluated by use of viral serologic testing and fecal *Helicobacter* PCR analysis at PIW 13 to determine whether airborne transmission had occurred.

Exhaust air filters. Pieces of sterile rayon/polyester gauze were placed on exhaust prefilters to determine the stability of viral/bacterial nucleic acids and the turnover of infectious agents on gauze filters under experimentally defined conditions. At the start of the experiment, 6 gauze filters were placed in the exhaust filter port of the IVC rack, on the inner surface of the prefilter upstream of the HEPA filter. The placement of six 1-cm^2 gauze filters, with pore size of approximately 1 mm^2, on the 50-cm^2 prefilter did not significantly decrease airflow in the rack (data not shown). One gauze filter was supposed to be removed from the prefilter at PIW 2, 4, 6, 8, 10, and 12, but all gauze filters were accidentally removed at PIW 2. Four gauze filters were replaced on the prefilter at PIW 4, and one gauze filter was then removed at PIW 6, 8, 10, and 12. A second set of gauze filters was used to determine when each infectious agent was detectable by use of PCR analysis. A sterile gauze filter was placed on the inner surface of the same exhaust filter port for a 24-h period at PIW 2, 4, 6, 8, 10, and 12. Particulate material was rinsed off the gauze filters using 500 µl of phosphate-buffered saline, nucleic acids were extracted, and MHV, MPV, murine rotavirus, SV and *H. muridarum* nucleic acids were detected by use of PCR or RT-PCR analysis.

Timed positive-control bedding exposure (group D). Twenty-four mice housed in 6 cages were used to monitor soiled bedding from index mice for infectious agents progressively throughout the experiment (Fig. 1A, cages D1-D6). These mice were used to determine if and when soiled bedding could transmit an infectious agent. Two ounces of soiled bedding was removed and pooled from each cage of index mice (20 cages) by a research technician who was not blinded as to which cages contained index mice. Each group of 4 positive-control bedding exposure mice received pooled soiled bedding from index mice once. Mice in cage D1 received soiled bedding from index mice at PIW 1, cage D2 at PIW 2, cage D3 at PIW 4, cage D4 at PIW 6, cage D5 at PIW 8, and cage D6 at PIW 10. Transmission of infectious agents was detected using viral serologic testing and fecal *Helicobacter* PCR analysis at PIW 13.

Uninoculated mice. Ninety-six mice were placed in 24 of the remaining cages (Fig. 1A; U cages) of the IVC rack to serve as controls to confirm that infectious agents were not accidentally transmitted between cages during husbandry operations. At PIW 13, uninoculated mice were evaluated by use of viral serologic testing and fecal *Helicobacter* PCR analysis to confirm lack of infectious agent transmission.

Design of negative air pressure experiment. Most aspects of the design of the second experiment were similar to those of the positive air pressure experiment, with several exceptions. The IVC rack was re-commissioned with a cage differential pressure of -1.5 ± 0.5 Pa. These values were confirmed to be uniform between all cages on the IVC rack. This airflow and differential pressure were maintained in the IVC rack throughout the experiment. The rack was configured as indicated in Fig. 1B. Since not all mice inoculated with MPV-1a in the first experiment became infected, an additional group of index mice was inoculated with MPV-1d for the second experiment. Because *H. muridarum*-infected mice did not transmit bacteria to soiled bedding sentinels in the first experiment, several changes were made to the *Helicobacter* part of the second experiment. The *Helicobacter* species that was used was changed to *H. hepaticus* as it has been documented to be transmitted by soiled bedding to mice housed in static cages with filter cage tops (12). Several of the BALB/c T-cell receptor alpha knockout mice naturally infected with *H. muridarum* of the first experiment developed severe rectal prolapse, and euthanasia was required.

In the second experiment, *H. hepaticus*-infected Swiss Webster index mice were used. Gauze filters were placed in the exhaust filter port on the inner surface of the prefilter upstream of the HEPA filter at the initiation of the experiment. These gauze filters were intended to monitor the stability of infectious agents on the gauze filters and were removed at PIW 1, 2, 3, 4, and 6. Gauze filters were also placed on the prefilter for 24-h periods at PIW 1, 2, 3, 4, and 6. Finally, gauze filters were also placed on the exterior of the cage lid exhaust filter of 10 IVC cages housing index mice (2 cages per infectious agent; Fig. 1B, cages marked with asterisks) for 24-h periods at PIW 1, 2, 3, and 4 to monitor the timing of release of each agent into the exhaust air stream.

Transmission of *H. hepaticus* in static cages with filter cage tops. To investigate the hypothesis that the high rate of air changes in an IVC cage, compared with the low rate of air changes in static cages with filter cage tops, might dry out the feces and affect the soiled bedding transmission of *H. hepaticus*, soiled bedding transmission of *H. hepaticus* from eight naturally *H. hepaticus*-infected mice housed in two static cages with filter cage tops to eight non-infected mice housed in two additional static cages with filter cage tops was measured. Two ounces of soiled bedding from each cage housing the naturally *H. hepaticus*-infected mice was mixed with 36 ounces of soiled bedding from static cages with filter cage tops housing uninoculated mice, then was transferred at PIW 2, 4, 6, 8, and 10 to two static cages with filter cage tops housing eight sentinel mice. A 20-fold dilution of soiled bedding was used to mirror the 20-fold dilution of soiled bedding added to the soiled bedding sentinel cages on the IVC rack.

Results

Positive air pressure experiment. All 12 index mice inoculated orally with MHV-Y developed subclinical infection and were MHV seropositive at PIW 2 and 13 (Table 2). Mouse hepatitus virus was transmitted to all contact, soiled bedding, and air sentinels (groups C, B, and Al; Table 2). Excretion of MHV RNA in feces of the index mice and contact sentinels was detected by use of RT-PCR analysis at PIW 2, 4, and 6 (Table 3). The duration of MHV transmission in soiled bedding was measured using timed positive-control soiled bedding exposure mice (group D), which received a single dose of soiled bedding pooled from all of the index mouse cages. Group-D mice that received soiled bedding at PIW 1, 2, or 4, but not those that received soiled bedding at later times, seroconverted to MHV (Table 4). The duration of MHV airborne transmission was measured using short-term exhaust air sentinels (group As) and gauze filters placed on the air exhaust prefilter of the IVC rack. Short-term

Table 2. Serologic test results for index and sentinel mice in the positive air pressure experiment

Mice	PIW	MHV	SV	EDIM	MPV-1a
Index mice	2	12/12[a]	10/10	12/12	7/12
	13	12/12	10/10	12/12	10/11
Contact sentinels	2	4/4	3/3	4/4	1/4
	13	4/4	3/3	4/4	3/4
Bedding sentinels[b]	4	8/8	0/8	0/8	0/8
	13	8/8	0/8	0/8	0/8
Air sentinels[c]	4	4/4	4/4	0/4	0/4
	13	4/4	4/4	0/4	0/4

[a]No. of seropositive index mice/total no. of index mice tested.
[b]Bedding sentinels were housed in cages marked B in Fig. 1A.
[c]Air sentinels were housed in the cage marked A1 in Fig. 1A.
PIW = postinoculation week; EDIM = murine rotavirus, the agent of epizootic diarrhea of infant mice.

Table 3. PCR and RT-PCR results for index and sentinel mice in the positive air pressure experiment

Mice	PIW	MHV[a]	EDIM[a]	MPV-1a[b]	*H. muridarum*[b]
Index mice	2	12/12	0/12	9/12	7/7
	4	4/11	0/12	1/10	.....
	6	1/5	0/12	0/12	.....
	13	.....	.....	.....	4/4
Contact sentinels	2	4/4	0/4	1/4	7/7
	4	2/4	0/3	3/4	6/6
	6	0/4	.....	2/4	6/7
	13	.....	.....	.....	8/8
Bedding sentinels[c]	4	.....	.....	.....	0/8
	13	.....	.....	.....	0/8
Air sentinels[d]	4	.....	.....	.....	0/4
	13	.....	.....	.....	0/4

[a]No. of fecal specimens that were RT-PCR positive/total no. of fecal specimens tested.
[b]No. of fecal specimens that were PCR positive/total no. of fecal specimens tested.
[c]Bedding sentinels were housed in cages marked B in Fig. 1A.
[d]Air sentinels were housed in the cage marked A1 in Fig. 1A.
..... = Not determined.

Table 4. Serologic test and molecular results for timed positive-control soiled bedding mice used to determine when infectious agents could be transmitted in soiled bedding from index/contact sentinel mouse cages[a]

PIW[b]	MHV[c]	SV[c]	EDIM[c]	MPV[c]	*Helicobacter* sp.[d]
Positive air pressure experiment					
1	4/4	0/4	0/4	4/4	0/4
2	4/4	0/4	0/4	4/4	0/4
4	4/4	0/4	0/4	0/4	0/4
6	0/4	0/4	0/4	0/4	0/4
8	0/4	0/4	0/4	0/4	0/4
10	0/4	0/4	0/4	0/4	0/4
Negative air pressure experiment					
1	4/4	1/4	.....	4/4	0/4
2	4/4	0/4	.....	4/4	0/4
4	4/4	0/4	.....	4/4	0/4
6	0/4	0/4	.....	4/4	0/4
8	0/4	0/4	.....	0/4	0/4
10	0/4	0/4	.....	0/4	0/4

[a]Mice of the positive-control soiled bedding group were housed in cages marked as D1–D6 in Fig. 1.
[b]Postinoculation week when soiled bedding from index mice was added to the cage.
[c]No. of seropositive mice/total no. of mice tested.
[d]No. of fecal specimens that were PCR positive/total no. of fecal specimens tested.
See Table 3 for key.

exhaust air sentinels (As) placed in a BioScreen cage between PIW 0 and 4 seroconverted to MHV, whereas those placed in this cage between PIW 4 and 12 did not seroconvert to MHV (Table 5). Gauze filters placed on the prefilter for 24 h from PID 17 through 55 were MHV-RNA positive (Table 6). Collectively, these data indicate that MHV was transmissible by contact, soiled bedding, and air for approximately 4 weeks in mice housed in the IVC.

Table 5. Serologic test and molecular results for short-term exhaust air sentinel mice used to determine the duration of airborne transmission[a]

Exposure period[b]	MHV[c]	SV[c]	EDIM[c]	MPV[c]	*Helicobacter* sp.[d]
Positive air pressure experiment					
0-2	4/4	4/4	0/4	0/4	.....
2-4	4/4	0/4	0/4	0/4	.....
4-6	0/4	0/4	0/4	0/4	0/4
6-8	0/4	0/4	0/4	0/4	0/4
8-10	0/4	0/4	0/4	0/4	0/4
Negative air pressure experiment					
0-2	0/4	4/4	.....	0/4	0/4
2-4	0/4	0/4	.....	0/4	0/4
4-6	0/4	0/4	.....	0/4	0/4
6-8	0/4	0/4	.....	0/4	0/4
8-10	0/4	0/4	.....	0/4	0/4

[a]Short-term exhaust air sentinels were housed in the cage marked As in Fig. 1.
[b]Postinoculation weeks that mice were exposed to exhaust air.
[c]No. of seropositive sentinel mice/total no. of sentinel mice tested.
[d]No. of fecal specimens from sentinel mice that were PCR positive/total no. of fecal specimens tested.
See Table 3 for key.

Table 6. PCR and RT-PCR results for individually ventilated caging (IVC) exhaust air filters in the positive air pressure experiment

PID[a]	MHV[b]	SV[b]	EDIM[b]	MPV-1a[c]	*H. muridarum*[d]
Filters exposed to air for 1 day[e]					
17-18	+	+	-	-	+
27-28	+	-	-	-	-
41-42	+	-	-	-	-
55-56	+	-	-	-	-
69-70	-	-	-	-	-
83-84	-	-	-	-	-
Filters exposed to air for multiple days[e]					
1-13	+	+	-	+	+
27-41	+	+	-	-	+
27-55	+	-	-	-	+
27-69	+	-	-	-	+
27-83	-	-	-	-	+

[a]Days after inoculation when gauze filter was present on the IVC exhaust prefilter.
[b]RT-PCR result for RNA extracted from gauze filter.
[c]PCR result for DNA extracted from gauze filter.
[d]Filters tested to document the temporal aspect of infectious agent shedding.
[e]Filters tested to document the relative stability of nucleic acids for each infectious agent.

The 12 mice inoculated intranasally with SV developed hunched posture and ruffled fur beginning on day 6, and 2 mice were found dead in their cage at days 8 and 12. The remaining 10 SV-inoculated mice were SV seropositive at PIW 2 and 13 (Table 2). Sendai virus was transmitted to all contact and air sentinels (groups C and Al), but seroconversion was not detected in the soiled bedding sentinels (group B) at any time (Table 2). One contact sentinel was found dead in its cage on day 14. Excretion of SV RNA from the index mice and contact sentinels was detected by use of RT-PCR analysis of cage swab specimens at PIW 2, but not at later times. None of the positive-control soiled bedding exposure mice (group D) seroconverted to SV (Table 4). Short-term exhaust air sentinels (As) placed in a BioScreen cage between PIW 0 and 2 seroconverted to SV, whereas those placed in this cage at later times did not seroconvert to SV (Table 5). The gauze filter placed on the prefilter for 24 h at PID 17 was SV RNA positive (Table 6). Collectively, these data indicate that SV was transmissible by contact and air for approximately 2 weeks.

All 12 index mice inoculated orally with murine rotavirus developed subclinical infection and were murine rotavirus seropositive at PIW 2 and 13 (Table 2). Murine rotavirus was transmitted to contact sentinels (group C), but not to soiled bedding or air sentinels (groups B and Al; Table 2). Excretion of murine rotavirus in feces of the index mice and contact sentinels was not detected by use of RT-PCR analysis (Table 3). None of the group-D soiled bedding exposure mice seroconverted to murine rotavirus (Table 4). Sentinels placed in BioScreen cage As between PIW 0 and 12 did not seroconvert to murine rotavirus (Table 5). Gauze filters placed on the prefilter for 24 h at various times were negative for murine rotavirus RNA (Table 6). Collectively, these data indicate that murine rotavirus was transmissible by contact only.

The 12 index mice inoculated oronasally with MPV-1a developed subclinical infection. Seven of 12 MPV index mice at PIW 2 and 10 of 11 MPV index mice at PIW 13 were MPV seropositive (Table 2). Mouse parvovirus-1a was ineffectively transmitted to contact sentinels; one contact sentinel was seropositive for MPV at PIW 2, and an additional two contact sentinels became seropositive for MPV by PIW 4 (Table 2). The fourth contact sentinel and all of the soiled bedding and air sentinels did not seroconvert to MPV (Table 2). Mouse parvovirus DNA was detected in 0 to 75% of fecal specimens from index mice and contact sentinels tested at PIW 2, 4, and 6 (Table 3). Group-D mice that received soiled bedding pooled from all of the index mouse cages at PIW 1 or 2, but not those that received bedding at later times, seroconverted to MPV (Table 4). Long-term and short-term exhaust air sentinels (groups Al and As) placed in a BioScreen cage between PIW 0 and 12 did not seroconvert to MPV (Tables 2 and 5). Gauze filters placed on the prefilter for 24 h at various times were negative for MPV DNA (Table 6). Collectively, these data indicate that MPV was excreted in feces for approximately 4 weeks and was inefficiently transmitted by contact and concentrated soiled bedding.

The 8 mice inoculated by contact with *H. muridarum*-infected mice developed subclinical infection, and *Helicobacter* DNA was detected in the feces of all contact sentinels between PIW 2 and 13 (Table 3). *H. muridarum* was not transmitted to soiled bedding or air sentinels (groups B , Al, and As; Tables 3 and 5). Group-D mice that received a single dose of soiled bedding pooled from all of the index mouse cages did not have detectable *Helicobacter* DNA in the feces at any time (Table 4). A gauze filter placed on the prefilter for 24 h at PID 17 but not at later times was *Helicobacter* DNA positive by PCR (Table 6). Collectively, these data indicate that *H. muridarum* was excreted in the feces of inoculated mice throughout the 12 weeks, but was transmissible only by contact.

All index mice and their corresponding contact sentinels were seronegative for the viruses with which they were not inoculated, and virus-inoculated mice were seronegative for *Helicobacter* DNA by use of PCR analysis. All 96 uninoculated mice were seronegative for MHV, SV, murine rotavirus, and MPV, and were *Helicobacter* DNA negative by use of PCR analysis.

To determine the stability of viral and bacterial nucleic acids and the turnover of infectious agents on gauze filters under experimentally defined conditions, 6 gauze filters were placed on the exhaust air prefilter of the IVC rack at initiation of the experiment. One gauze filter was supposed to be removed from the prefilter at PIW 2, 4, 6, 8, 10, and 12. All gauze filters were accidentally removed at PIW 2 when accumulated particulates were shaken off of the prefilter. Mouse hepatitus virus, SV, MPV, and *Helicobacter* sp., but not murine rotavirus, were detected by use of RT-PCR and PCR analysis of the gauze filter present on the IVC rack from PIW 0 through 2 (Table 6). Four gauze filters were replaced on the prefilter at PIW 4 and one gauze filter was removed at PIW 6, 8, 10, and 12. Murine rotavirus RNA and MPV DNA were not detected on any of the gauze filters collected at PIW 6, 8, 10, and 12 (Table 6). Sendai virus RNA was detected on the gauze filter removed at PIW 6, MHV RNA was detected on the gauze filters removed at PIW 6, 8, and 10, and *Helicobacter* DNA was detected on the gauze filters at PIW 6, 8, 10, and 12 (Table 6).

Table 7. Serologic test results for index and sentinel mice in the negative air pressure experiment

Mice	PIW	MHV[a]	SV[a]	MPV-1a[a]	MPV[a]	MPV-1d[a]
Index mice	2	12/12	12/12	5/12		12/12
	13	12/12	12/12	7/12		12/12
Contact sentinels	2	4/4	4/4	1/4		3/4
	13	4/4	4/4	1/4		4/4
Bedding sentinels[b]	4	8/8	0/8		3/8	
	13	8/8	0/8		8/8	
Air sentinels[c]	4	4/4	4/4		0/4	
	13	4/4	4/4		0/4	

[a]No. of seropositive sentinel mice/total sentinel mice tested.
[b]Bedding sentinels were housed in cages marked B in Fig. 1B.
[c]Air sentinels were housed in the cage marked A1 in Fig. 1B.

Table 8. PCR and RT-PCR results for sentinel mice in the negative air pressure experiment

Mice	PIW	MHV[a]	MPV-1a[b]	MPV-1d[b]	*H. hepaticus*[b]
Index mice	2	12/12	2/8	6/8	7/7
	4	9/11	.....	1/8	8/8
	6	3/11	2/8	2/9	8/8
	13	0/8	0/4	2/8	.....
Contact sentinels	2	4/4	1/4	4/4	6/8
	4	4/4	1/4	4/4	5/8
	6	1/4	1/4	2/4	4/8
	13	0/4	0/4	2/4	4/8
Bedding sentinels[c]	4	.....	.....	.....	4/8
	6	.....	.....	.....	4/8
	13	.....	.....	.....	0/8
Air sentinels[d]	4	.....	.....	.....	0/8
	13	.....	.....	.....	0/8

[a]No. of fecal specimens that were RT-PCR positive/total fecal specimens tested.
[b]No. of fecal specimens that were PCR positive/total fecal specimens tested.
[c]Bedding sentinels were housed in cages marked B in Fig. 1B.
[d]Air sentinels were housed in the cage marked A1 in Fig. 1B.
See Table 3 for key.

Negative pressure experiment. All 12 index mice inoculated orally with MHV-Y developed subclinical infection and were MHV seropositive at PIW 2 and 13 (Table 7). Mouse hepatitus virus was transmitted to all contact, soiled bedding, and air sentinels (groups C, B and Al; Table 7). Mouse hepatitus virus RNA was detected in the feces of index mice and contact sentinels at PIW 2, 4, and 6 (Table 8). Positive-control soiled bedding exposure mice (group D) that received soiled bedding at PIW 1, 2, or 4, but not those that received soiled bedding at later times, seroconverted to MHV (Table 4). The duration of MHV transmission in air was measured using short-term exhaust air sentinels (group As), gauze filters placed on the air exhaust prefilter of the IVC rack, and gauze filters placed on the exterior of the foam cage lid filters. Unexpectedly, short-term exhaust air sentinels (group As) did not seroconvert to MHV (Table 5). Gauze filters

Table 9. PCR and RT-PCR results for IVC exhaust air filters in the negative air pressure experiment

PID[a]	MHV[b]	SV[b]	MPV[c]	*H. hepaticus*[c]
Filters exposed to air for 1 day[d]				
6-7	+	+	-	-
13-14	+	-	-	-
20-21	+	-	-	-
27-28	+	-	-	-
41-42	+	-	-	-
Filters exposed to air for multiple days[e]				
1-6	+	+	-	-
1-13	+	-	+	-
1-20	+	+	+	-
1-27	+	-	+	-
1-41	+	+	+	-

[a]Days after inoculation when gauze filter was present on the IVC exhaust prefilter.
[b]RT-PCR result of RNA extracted from gauze filter.
[c]PCR result of DNA extracted from gauze filter.
[d]Filters tested to document the temporal aspect of infectious agent shedding.
[e]Filters tested to document the relative stability of nucleic acids for each infectious agent.

placed on the IVC rack prefilter for 24 h between PIW 1 and 6 were MHV RNA positive (Table 9). Gauze filters placed on the cage lid filter of cages housing MHV inoculated mice for 24 h between PIW 1 and 4 were MHV RNA positive (Table 10). Collectively, these data indicate that MHV was transmissible by contact, soiled bedding, and air for approximately 4 weeks.

The 12 mice inoculated intranasally with SV, using an inoculum diluted twofold from that used in the first experiment, had ruffled fur beginning on day 6, but recovered by day 10. All SV index mice were SV seropositive at PIW 2 and 13 (Table 7). Sendai virus was transmitted to all contact and air sentinels (groups C and Al), but seroconversion was not detected in the soiled bedding sentinels (group B; Table 7). Excretion of SV RNA from the index mice and contact sentinels was detected by use of RT-PCR analysis of cage swab specimens at PIW 2, but not at later times. One of 4 positive-control soiled bedding exposure mice (group D) that received a single dose of soiled bedding pooled from all of the index mouse cages at PIW 1 seroconverted, but mice that received soiled bedding at later times did not seroconvert to SV (Table 4). Short-term exhaust air sentinels (group As) placed in a BioScreen cage between PIW 0 and 2 seroconverted to SV, whereas those placed in this cage between PIW 2 and 12 did not seroconvert to SV (Table 5). A gauze filter placed on the prefilter for 24 h at PIW 1 was SV RNA positive by use of RT-PCR analysis (Table 9). Gauze filters placed on the cage lid filter of 1 of 2 cages housing SV index mice for 24 h at PIW 1 and 2 were SV RNA positive by use of RT-PCR analysis (Table 10). Collectively, these data indicate that SV was efficiently transmitted by contact and air for up to 2 weeks.

All 12 index mice inoculated orally with murine rotavirus did not become infected and were murine rotavirus seronegative at PIW 2, 4, and 13. Murine rotavirus was not detected in contact, soiled bedding, or air sentinels (data not shown).

The 12 index mice inoculated oronasally with MPV-1a developed subclinical infection. Five of 12 MPV-1a index mice housed in three cages seroconverted to MPV at PIW 2, and 7 of 12 mice seroconverted by PIW 13 (Table 7). The MPV-1a was ineffectively transmitted to contact sentinels; only one contact sentinel seroconverted to MPV (Table 7). Feces collected from inoculated mice and the corresponding contact sentinel in a single cage at PIW 2, 4, and 6 were MPV DNA positive (Table 8). All 12 index

Table 10. PCR and RT-PCR results for cage lid exhaust air filters in the negative air pressure experiment

PID[a]	MHV[b]	SV[b]	MPV-1a[c]	MPV-1d[c]	*H. hepaticus*[c]
6-7	2/2	1/2	2/2	2/2	0/2
13-14	2/2	1/2	0/2	1/2	0/2
20-21	2/2	0/2	0/2	1/2	0/2
27-28	2/2	0/2	0/2	0/2	0/2

[a]Days after inoculation when gauze filter was present on top of cages housing index mice.
[b]No. of filters positive for viral RNA by use of RT-PCR/total filters tested.
[c]No. of filters DNA positive by use of PCR/total filters tested.

mice inoculated oronasally with MPV-1d developed subclinical infection. All MPV-1d-inoculated index mice and contact sentinels were MPV seropositive at PIW 13 (Table 7). Excretion of MPV-1d DNA from index mice and contact sentinels was detected with variable efficiency in feces collected at PIW 2, 4, and 6 (Table 8). All soiled bedding sentinels (group B) were MPV seropositive at PIW 13 (Table 7). By contrast, none of the exhaust air sentinels (groups Al and As) seroconverted to MPV (Tables 5 and 7). Gauze filters placed on the IVC rack prefilter for 24 h at various times were negative for MPV DNA by use of PCR analysis (Table 9). Gauze filters placed on the cage lid exhaust filter of cages housing MPV-1a-inoculated mice at PIW 1 were MPV DNA positive (Table 10). Gauze filters placed on the cage lid exhaust filter of cages housing MPV-1d inoculated mice between PIW 1 and 3 were MPV DNA positive (Table 10). Positive-control soiled bedding exposure mice (group D) that received soiled bedding pooled from all of the index mouse cages at PIW 1, 2, 4, or 6, but not those that received bedding at later times, seroconverted to MPV (Table 4). Collectively, these data indicate that MPV-1a was excreted in feces for 6 weeks and was inefficiently transmitted by contact; MPV-1d was excreted in feces for 13 weeks and was efficiently transmitted by contact; and MPV, probably strain 1d, was transmitted by soiled bedding.

The eight index mice inoculated by contact with *H. hepaticus*-infected mice, housed in the IVC rack, developed subclinical infection. *Helicobacter* DNA was detected in the feces of all index mice at PIW 2, 4, and 6 and in the feces of most contact sentinels at all times (Table 8). *Helicobacter* DNA was detected in the feces of mice in one of the two soiled bedding sentinel cages at PIW 4 and 6, but not at later times (Table 8). None of the positive-control soiled bedding exposure mice (group D) had detectable *Helicobacter* DNA in the feces (Table 4). *Helicobacter* DNA was not detected in the feces of mice in the air sentinel cages (groups Al and As) at any time (Tables 5 and 8). Gauze filters placed on the IVC rack prefilter or on the lids of cages housing *H. hepaticus*-infected mice for 24 h at various times were *Helicobacter* DNA negative by use of PCR analysis (Tables 9 and 10). Collectively, these data indicate that *H. hepaticus* was excreted in the feces of index mice housed in the IVC rack throughout the 13 weeks and was transmissible by contact and soiled bedding.

To explore the possibility that air change rate might affect the soiled bedding transmission of *Helicobacter* sp., transmission via contact and soiled bedding was measured using eight mice naturally infected with *H. hepaticus* housed in two static cages with filter cage tops. The eight index mice developed subclinical infection. *Helicobacter* DNA was detected in the feces of all index mice and contact sentinels at PIW 6 and 13. *Helicobacter* DNA was not detected in the feces of mice in the two soiled bedding sentinel cages at PIW 4, 6, and 13. Collectively, these data indi-

cate that *H. hepaticus* was excreted in the feces of index mice housed in the static cages with filter cage tops throughout the 13 weeks and was transmissible by contact only.

Apart from three exceptions, index mice and their corresponding contact sentinels remained seronegative for the viruses with which they were not inoculated. Three MHV index mice and a single MHV contact sentinel in a single cage were seropositive for MPV at PIW 13. Also, 12 SV index mice and three SV contact sentinels in 3 cages were seropositive for MHV at PIW 13. Finally, all 16 MPV-1a index mice and MPV-1a contact sentinels were MHV seropositive at PIW 13. All virus-inoculated mice were negative for *Helicobacter* DNA by use of PCR analysis. All 80 uninoculated mice were seronegative for MHV, SV, murine rotavirus, and MPV, and were *Helicobacter* DNA negative by use of PCR analysis.

To determine the stability of viral and bacterial nucleic acids and the turnover of infectious agents on gauze filters under experimentally defined conditions, 6 gauze filters were placed on the exhaust air prefilter of the IVC rack at PID 1. Gauze filters were gently removed from the soiled prefilter and placed on a clean prefilter weekly. One gauze filter was removed from the prefilter at PIW 1, 2, 3, 4, and 6 for testing. Mouse hepatitis virus RNA, SV RNA, and MPV DNA were detected on the gauze filters removed from the IVC rack between PIW 1 and 6 (Table 9). In contrast, *Helicobacter* DNA was not detected on the gauze filters removed from the IVC rack at any time (Table 9).

Discussion

Uninoculated mice remained uninfected in the positive and negative air pressure experiments, indicating that the infections observed in the sentinel mice were not transmitted during routine husbandry procedures but rather by directed exposure of sentinels via air, bedding, or contact. Additionally, the air pressure differential under which the rack was operated, whether positive or negative, did not affect the ability of the IVC to contain the infectious agents. The efficacy of infectious agent transmission to sentinel mice was dependent on many factors. Clear correlation was seen between the route of infectious agent shedding and the efficacy of agent detection using the corresponding sentinel type. For example, respiratory tract shedding was detected most effectively by exhaust air sentinels, and fecal shedding was detected most effectively by soiled bedding sentinels. Also, the efficacy of detection was dependent on the infectious agent load to which the sentinel mice were exposed. Infectious agent load depended on concentration of agent shed, duration of shedding, and stability of the agent after it was shed. The frequency of exposure to an infectious agent (once, several times or continuously) also impacted the efficacy of infectious agent detection.

Mouse hepatitis virus was the most transmissible agent, in that it was transmitted to sentinel mice by contact, soiled bedding, and air in positive and negative air pressure experiments. These results concur with previous results that indicated that MHV is readily transmitted by contact, soiled bedding, and room air (8, 19). Shedding of MHV in feces of index mice was detected for 4-6 weeks. Similarly, MHV was detected for 4 weeks in exhaust air on cage lid gauze filters and in soiled bedding from MHV index cages in positive-control soiled bedding exposure mice (group D).

Mouse hepatitis virus particles, infective and non-infective, released into the IVC airstream from cages housing index mice and from cages housing sentinels that became infected during the experiment, were detected on gauze filters placed on the IVC rack exhaust prefilter. In the first experiment, gauze filters placed on the IVC rack exhaust prefilter trapped MHV RNA for 10 weeks. The design for servicing the prefilter in the first experiment, removal of the gauze filters from the prefilter weekly followed by shaking of the prefilter and replacement of the gauze filters on the prefilter, may have contributed to the detection of MHV RNA for a such a long period. Visually, it was clear that shaking of the prefilter was not effective at removing all particulates. Therefore, gauze filters placed back on the prefilter may have acquired MHV RNA from the MHV particles present in particulates on the prefilter surface rather than from the air. The IVC filters tested in the second experiment should have yielded more accurate results as to when MHV was present in the exhaust air since gauze filters were gently removed from the soiled prefilter on a weekly basis and placed on a clean autoclaved prefilter. However, since MHV was still being shed from several index mice and contact sentinels at PIW 6 and gauze filters were only collected from the IVC prefilter for 6 weeks, it was not feasible to determine the stability of MHV RNA on the gauze filters. Mouse hepatitis virus RNA would be expected to be stable for at least a week after excretion since one report involving rat coronavirus indicated that coronaviral RNA can be detected for at least 7 days after being deposited on a plastic surface (6). Additional studies will be necessary to determine the stability of MHV RNA deposited on gauze filters.

In both experiments, SV was effectively transmitted to sentinel mice by contact and air but not by soiled bedding. These results concur with previous results documenting that SV is efficiently transmitted by contact and room air (8). Sendai virus induced more acute infection than did MHV, and airborne SV shedding was detected by use of RT-PCR analysis of cage swab specimens or cage lid gauze filters and by transmission to mice of the short-term exhaust sentinel group for only 2 weeks. A recent report (20) indicated that SV RNA was only detectable for 3 days on the surface of cages housing SV-infected mice; however, we report that SV RNA was detectable on the cage surface for 2 weeks. Different methods of sampling the cage surface (e.g., alcohol swipes versus calcium alginate swabs moistened with saline) could account for this difference in time of detectability. Alternatively, presence of a contact sentinel in the cages, the more severe nature of SV disease, or use of outbred mice in our study, may have extended the period of SV deposition onto the cage surface. In the negative air pressure experiment, SV RNA was detected on the gauze filter placed on the IVC prefilter for 24 h at PIW 1, but not at later times, indicating that the amount of SV RNA in the air had decreased below that detectable by use of RT-PCR analysis by PIW 2. The sporadic nature of SV RNA detection on gauze filters placed on the prefilter at PID 1 and removed at weekly intervals suggests that the amount of SV RNA on the gauze filter may have been close to the limit of detection by use of RT-PCR analysis. The ability to detect SV RNA, presumably deposited on the gauze filter within the first week of infection, 2-5 weeks later indicates that the SV RNA was stable. Only a single mouse of the positive-control soiled bedding exposure group D that received concentrated soiled bedding from index mice at PIW 1, in the second experiment, seroconverted. This is consistent with reports that soiled bedding transmission

of SV occurs sporadically and is dependent on dose of virus, time after inoculation, and strain of sentinel mice (1). The most reliable method of detecting SV infection appears to be using exhaust air sentinels.

Infection with murine rotavirus was transmitted only to contact sentinels in the positive air pressure experiment. Excretion of murine rotavirus was not detected in feces collected at PIW 1 or later by use of RT-PCR analysis. The inability to initiate murine rotavirus infection in the negative air pressure experiment suggests that the dose of murine rotavirus used was probably close to the dose needed to initiate infection. To the authors' knowledge, the efficacy of soiled bedding sentinels for detecting murine rotavirus infection has not been investigated. The epidemiology of murine rotavirus infection in adult mice, with viral shedding in feces only between PID 2 and 7, would suggest that the use of biweekly soiled bedding transfers for detecting murine rotavirus infection would be an unreliable technique (21).

Results of both experiments indicated that MPV-1a was inefficiently transmitted to contact sentinels and was shed in the feces of inoculated mice for 4-6 weeks. The inability to infect all index mice with our stock of MPV-1a may have contributed to the inefficiency of MPV-1a transmission. Route of inoculation has been reported to influence the efficiency with which MPV infection is initiated, with combined intraperitoneal/oral inoculation being more efficient than oral inoculation alone (17). Additionally, mouse genotype affects the efficiency of MPV infection initiation; outbred mice are less susceptible to MPV infection than are C3H mice (2). The dilution of virus present in soiled bedding transferred to sentinels cages affected transmission of MPV infection to sentinels. For example, positive-control soiled bedding exposure mice (group D) that received a single dose of the more concentrated soiled bedding from cages housing index mice (MPV-inoculated mice in four of 20 cages) seroconverted, whereas those that received five doses of more dilute soiled bedding from all cages on the IVC rack (MPV-inoculated mice in one cage of 18 cages) did not seroconvert. This reinforces observations that detection of MPV infection in contemporary mouse colonies using soiled bedding sentinels is sporadic and is highly dependent on the extent of infection within a rack. For MPV and other agents for which shedding of the viral load is low, inadequate sampling of soiled bedding can result in inaccurate monitoring. Additional studies are needed to more accurately determine the most effective soiled bedding sampling protocol needed to reproducibly detect MPV infection in mice housed in IVC.

In contrast to MPV-1a, inoculation of mice with our stock of MPV-1d resulted in chronic infection of all index mice, with shedding of virus in the feces through the entire experimental time period. The transmission of MPV by soiled bedding transfer observed in the negative air pressure experiment was probably the result of MPV-1d infection. In that experiment, MPV DNA was not detected on the gauze filters placed on the IVC prefilter for a single day, but was detected on gauze filters placed on the IVC prefilter for two weeks or longer, indicating that the amount of MPV DNA being deposited on the gauze filters was low and an extended period was need to accumulate detectable amounts of MPV DNA. By contrast, a single day of exposure of gauze filters placed directly on the cage lids of MPV-1d-inoculated mice resulted in accumulation of detectable amounts of MPV-1d DNA. The MPV DNA detected on the gauze filters placed on the IVC prefilter was probably MPV-1d DNA since it was detected more frequently than was MPV-1a DNA on cage lid gauze filters. In performing these experiments, we were not able to determine the stability of MPV DNA on gauze filters as MPV-1d shedding persisted throughout the experiment, but results of a previous study indicated that rat parvovirus dried on a plastic surface was infective after storage at room temperature for 3-5 weeks (24). It is likely that MPV deposited on the gauze filters should also be stable for several weeks.

In the positive air pressure experiment, *H. muridarum* was transmitted to all contact sentinels and they shed bacteria in the feces throughout the study. *Helicobacter muridarum* was not transmitted to soiled bedding sentinels. Several murine *Helicobacter* species (*H. hepaticus, H. bilis, H. rodentium*) have been reported to be transmissible to soiled bedding sentinels but, to our knowledge, soiled bedding transmission of *H. muridarum* infection has not been documented (12, 22). Although exhaust air sentinels did not become infected, *H. muridarum* was detected on gauze filters placed on the IVC prefilter, indicating that *H. muridarum* DNA was present in the exhaust air but that the amount of infective *H. muridarum* was below that necessary to initiate infection.

In the negative air pressure experiment, *H. hepaticus* was transmitted to contact sentinels and they shed the organism in the feces throughout the experiment. *Helicobacter hepaticus* was inefficiently transmitted to sentinels that received five doses of soiled bedding from all mice housed on the IVC rack. Mice that received five doses of diluted soiled bedding from *H. hepaticus*-infected mice housed in static cages with filter cage tops did not become infected. Additionally, transmission did not occur after transfer of a single concentrated dose of soiled bedding from the IVC cages housing index mice and contact sentinels. Our theory that the drying out of soiled bedding in the IVC, compared with the moister soiled bedding in static cages with filter cage tops, might impair soiled bedding transmission of *Helicobacter* species was not substantiated. Additional studies will be necessary to clarify whether environmental differences such as humidity, which differ between IVC and static cages with filter cage tops, impact the infectious load of *Helicobacter* species and other infectious agents and, therefore, the efficacy of various monitoring methods in these housing systems.

The lower efficiency of transmission of *H. hepaticus* observed in our experiments in which mice were exposed to soiled bedding mixed from cages housing infected and uninfected mice biweekly, compared with that of a previous study where mice were exposed twice a week to soiled bedding from cages housing only infected mice, can probably be explained by these differences in the frequency of soiled bedding transfer and the concentration of helicobacters present in the soiled bedding (12). *Helicobacter hepaticus* was not detected in the exhaust air using IVC rack prefilter or cage lid gauze filters or exhaust air sentinels, even though *H. hepaticus* has been reported to be transmitted, presumably in the air, between mice housed in open-top cages (9).

The use of gauze filters placed on the prefilter to monitor the exhaust air from all cages on the rack or on cage lids to monitor individual cages could greatly improve a clinician's ability to determine whether and to what extent an agent is being shed into the air. It can be used to accurately locate an infection to a particular rack within a room or to a particular cage on a rack without the need to handle animals. This can be valuable during an

outbreak when handling animals (e.g., for blood sample or soiled bedding collection, or other experimental manipulations) may lead to cross contamination. Additionally, monitoring exhaust air filters can be performed with a minimum of labor and can provide results in less than 2 days.

When infections are present in animals of only a small number of cages on an IVC rack (as in these experiments and in many endemic infections with infectious agents of low transmissibility), reliability of exhaust air monitoring using gauze filters placed on the rack prefilter or using exhaust air sentinels is likely to be highly dependent on uniformity of the airflow within the rack. Non-uniform airflow could lead to inaccurate sampling of exhaust air from the cages housing infected mice. The optimal position for filter placement within the rack that will result in the most accurate sampling will need to be determined for each IVC rack system. An important advantage of exhaust air sentinel monitoring is that it monitors all cages all the time. In contrast, the soiled bedding monitoring method samples a limited number of cages at each cage change so that any one cage may only be sampled once every 2-3 months. For infectious agents that are shed only over a short time interval, continuous monitoring could be critical for detection. Only a single BioScreen cage of long-term exhaust air sentinels was used in this study. It is possible that use of a second such cage could increase the likelihood that agents being shed at low amounts into the exhaust air will be detected.

Short-term exhaust air sentinels, which were used in this study to monitor the time frame during which each infectious agent was transmissible in the exhaust air, gave variable results. As expected, short-term exhaust air sentinels exposed to exhaust air between PIW 0 and 2 in the positive and negative air pressure experiments seroconverted to SV. By contrast, short-term exhaust air sentinels in the positive, but not the negative air pressure experiment, seroconverted to MHV. We can be confident that the airflow in the negative air pressure experiment was moving correctly since the same short-term exhaust air sentinels seroconverted to SV. Also infective MHV was present in the exhaust air because the long-term exhaust air sentinels in the same study seroconverted to MHV.

In the negative air pressure experiment, cross infection with MHV and MPV was observed between inoculated mice in the index cages. Cross infection most likely occurred during removal of soiled bedding by a research technician from the index cages at PIW 1 or 2 (when soiled bedding was documented to contain infective MHV and MPV) for placement in the cages containing positive-control bedding exposure mice (group D). Since the uninoculated mice on the rack remained seronegative for all viruses, it seems that routine cage changes performed by the animal caretaker (who was blinded to the experimental setup) were not the source of this cross contamination. This serves as a warning that when virus load is at its peak, the experimental manipulation of mice, even if performed carefully, can lead to cross contamination of mice. The clear difference in the ease with which agents were transmitted in this study gives us a good indication of the impact of IVC on intra-colony spread of infections. Mouse hepatitis virus can be inadvertently transmitted between cages due to even minor mistakes during the peak of viral shedding. On the other hand, agents like murine rotavirus, or to some degree MPV, are difficult to transmit between cages even intentionally. It can be speculated that even if cage manipulations are performed suboptimally, the likelihood of these infections spreading through a mouse colony maintained in IVC is low. Since SV seems to require either direct contact or airborne exposure for transmission and causes short-lived infection in immunocompetent mice, it is unlikely that this virus can sustain itself in a mouse colony housed in IVC regardless of husbandry practices used.

In conclusion, all methods were effective at detecting MHV infection and SV was effectively detected only by exhaust air sentinels. Mouse parvovirus and *Helicobacter* spp. were transmitted in soiled bedding, but the efficacy of soiled bedding transfer was dependent on the frequency and dilution of soiled bedding transferred. No single method of detection was fully reliable for all organisms tested. This makes the design of an effective monitoring program challenging; therefore, each program should be based on risk assessments of encountering the panel of infectious agents of interest and the statistical likelihood of detecting these agents when they are present. Results were similar when the IVC rack was operated under positive and negative air pressure and, in general, results from our IVC experiments were similar to those obtained by previous investigators using static cages with filter cage tops. Gauze filters were found to be highly effective at detecting MHV and SV and were less effective at detecting MPV. Viral nucleic acids were detected on gauze filters for at least a month, indicating that they are quite stable. These results indicate that exposure of sentinel mice or gauze filters to exhaust air is effective at detecting several infectious agents and use of these methods could increase the efficacy of microbiological monitoring programs, especially if used in conjunction with soiled bedding transfer. Because exhaust air monitoring methods are less labor-intensive, and therefore more cost-efficient than are traditional soiled bedding monitoring, the judicious use of exhaust air monitoring could reduce animal use charges. In contemporary mouse colonies where, due to movement of mice between institutions and the use of a wide array of genetically engineered and often immunodeficient mice, the risks of exposure of mice to infectious agents is increasing, a multi-faceted approach to microbiological monitoring is necessary.

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