Journal of the American Association for Laboratory Animal Science
Vol 51, No 4
July 2012
Pages 436–442
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Prevalence of Murine *Helicobacter* spp. Infection Is Reduced by Restocking Research Colonies with *Helicobacter*-Free Mice

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Most academic research colonies of mice are endemically infected with enterohepatic *Helicobacter* spp. (EHS). We evaluated EHS prevalence in surveillance mice before and after a 10-y period of requiring that imported mice be free of EHS by embryo transfer rederivation or purchase from approved vendors. In 2009, composite fecal samples from CD1 surveillance mice representing colony health in 57 rooms located in 6 facilities were evaluated for EHS infection by using PCR assays. Fecal samples were screened with primers designed to detect all known EHS, and positive samples were further assayed by using primers specific for *H. hepaticus*, *H. bilis*, *H. rodentium*, and *H. typhlonicus*. Most EHS were detected in surveillance mice within the first month of dirty bedding exposure, with prevalence ranging from 0% to 64% as monoinfections or, more commonly, infections with multiple EHS. Compared with 1999 prevalence data, EHS remained endemic in colonies importing the lowest number of EHS-free mice. EHS were absent or the prevalence was greatly reduced in colonies receiving the highest percentage of EHS-free mice. This study demonstrates that the management decision to require exclusive importation of EHS-free mice reduced EHS prevalence on an institutional scale without intensive labor and expense associated with other techniques or interference with research objectives.

Abbreviations: EHS, enterohepatic *Helicobacter* spp.; ET, embryo transfer; Hb, *H. bilis*; Hh, *H. hepaticus*; Hm, *H. mastomyrinus*; Hr, *H. rodentium*; Ht, *H. typhlonicus*.

Enterohepatic *Helicobacter* spp. (EHS) infections are endemic in the majority of research mouse colonies. In 2007, 84% of mice shipped from academic institutions worldwide for embryo transfer (ET) rederivation at our institution were PCR-positive for EHS. *H. hepaticus* (Hh) was detected in 64% of the mouse shipments either as a monoinfection or in combination with other EHS including *H. bilis* (Hb), *H. rodentium* (Hr), *H. typhlonicus* (Ht), and *H. mastomyrinus* (Hm).[30] Although EHS generally cause subclinical infection in immunocompetent mice, opportunistic infections have the potential to confound experimental data in mouse models.[9,17,34] Importantly, chronic EHS infection in immunodeficient and select inbred strains of mice can induce liver[10] and lower bowel carcinoma,[13] typhlocolitis, and rectal prolapse,[16,21,28] and reduce reproductive performance.[25] In addition, EHS-induced inflammatory responses may alter host immune responses to unrelated experimental infections (for example, promoting elevated systemic IFNγ responses).[3,20]

Key challenges to eradication of EHS from rodent colonies are determining infection status, eliminating endemic infections, and instituting management practices that prevent reinfection. EHS are disseminated through fecal–oral transmission within a colony and are transmissible to surveillance mice through dirty-bedding exposure.[1,19,24,32] For routine surveillance, PCR assay of feces or cecal mucosal scrapings for genus-specific *Helicobacter* 16S rRNA genes is the most efficient means of detecting EHS infection, with speciation (if desired) of positive results by culture, restriction fragment length polymorphism analysis, species-specific PCR, or sequence analysis.[34] In 1999, as determined by species-specific PCR assays of cecal scrapings from 59 surveillance mice exposed to dirty bedding from colony mice in 26 rooms representing 4 mouse facilities, EHS were endemic on our campus, with prevalence in surveillance mice of 41% for Hh, 82% for Hr, and 6% for Hb.[32] Husbandry practices used to minimize cage-to-cage transmission of EHS included microisolation caging, sanitized forceps to transfer mice, and a cage change order from known *Helicobacter*-free mice to mice of unknown or known EHS infection status (that is, clean to dirty traffic flow of personnel and equipment).[32] Although EHS eradication potentially could be accomplished campus-wide by using labor-intensive antibiotics[7,15] and cross-fostering,[4,29,31] we hypothesized that a more cost-effective approach, without confounding experimental data, would be to restrict importation of mice to EHS-free sources. Vendors were screened to establish that production colonies were SPF for EHS, and a new requirement was instituted for embryo transfer (ET) rederivation of mice obtained from random sources, typically other academic institutions, replacing traditional quarantine practices. This study used PCR data from 1999 and 2009 to evaluate the success of this approach, which was defined as a marked decrease in the prevalence of EHS infection over time.

Materials and Methods

Methods were conducted as part of the murine health surveillance program as approved by the Massachusetts Institute of Technology Committee on Animal Care.

Facilities surveyed. Each of the 6 facilities surveyed in 2009 differed in the level of investigator access, the number of

Received: 21 Jul 2011. Revision requested: 24 Aug 2011. Accepted: 09 Dec 2011.
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ET-rederived mice, and the extent of breeding within relatively closed colonies to obtain mice for experiments (Table 1). All facilities were restricted by card access to investigators that had completed facility-specific orientation and were aware of the 'no return' policy for mice taken out of the facilities. Only facility F allowed mice to be returned from survival procedures conducted in laboratories within the same building, but these mice were housed in a designated return room. Personnel needing to access multiple facilities during the same work day were required to travel in the A-through-F sequence (Table 1) or to shower if another entrance order was critical.

All facilities required sanitization of materials originating from outside of the facility prior to entry; this was primarily achieved by spraying with Quatracide PV (Pharmacal Research Laboratories, Naugatuck, CT) or alternative methods as necessary. Husbandry and laboratory personnel were required to wear facility-specific laboratory coats, disposable shoe and head covers and gloves when handling mice. Facility A consisted of a physically separate barrier hallway with access restricted to select Division of Comparative Medicine staff involved in producing transgenic, knockout, and ET-rederived mice. The designated staff working in this barrier were required in addition to wear one-use disposable lab coats, face masks, and gloves at all times. A second housing area in facility A was accessible to investigators by using ET-rederived experimental mice or mice purchased *Helicobacter*-free from approved vendors. Facility B is described as a semibarrier (Table 1) because it exclusively housed genetically modified and wildtype mice either produced within the facility A barrier or were purchased *Helicobacter*-free. Facility B mice belonged to a single large laboratory and were used in behavioral test spaces integrated within the facility. Facilities C and D predominately housed mice used by long-term investigators that bred most of their experimental mice for use in the same facility and therefore had comparatively lower requirements for purchasing additional mice. These facilities imported vendor mice or ET-rederived mice as needed, but no housing decisions were made based on the EHS infection status of the receiving colony. Facilities E and F housed smaller colonies of either ET-derived or vendor-sourced EHS-free mice for experimental use, with minimal breeding.

ET rederivation program. Mice received from random, typically academic, sources were bred in a separate quarantine facility only long enough to produce embryos that were collected postmortem and hand-delivered to the facility A barrier for surgical transfer into *Helicobacter*-free, barrier-maintained, recipient CD1 mice. The *Helicobacter*-free status of recipient female and vasectomized male mice in facility A was monitored by *Helicobacter* genus-level PCR assay of cecal scrapings from euthanized recipient female mice after weaning of their pups. In addition, surveillance CD1 mice received dirty bedding from the rederivation support colony.

EHS-free vendors. Purchase orders for mice were restricted to several major vendors providing *Helicobacter*-free mice. The SPF status for EHS was confirmed periodically by veterinary review of vendor barrier status and inhouse testing of mice for *Helicobacter* spp. by using PCR.

Husbandry practices. All facilities were centrally managed under the auspices of an AAALAC-accredited program. Standard environmental conditions were 22 ± 1 °C, 30% to 70% humidity, 10 to 15 room air changes hourly, and a 12:12-h light:dark cycle. Colony mice were housed on either static or ventilated racks in polycarbonate microisolation cages with filter tops (Allentown, Allentown, NJ) at a density of 3 to 5 mice in small (7.5 × 11.5 × 5 in.) cages or 6 to 10 mice in large (10.5 × 19 × 6 in.) cages. Heat-treated hardwood bedding (Beta Chip, Nepco, Warrensburg, NY) and cotton nesting pads (Nestlets, Ancare, Bellmore, NY) were provided in addition to ad libidum pelleted diet (RMH 3000, Purina Mills, Richmond, IN) and filtered tap or reverse-osmosis water. Mice were transferred from dirty to clean cages by using metal forceps briefly dipped between cages in Quatracide PV (Pharmacal Research Laboratories) diluted 1:64. Dirty cages were sanitized by using detergent (Clout, Pharmacal Research Laboratories) with 180 °F rinse water verified by indicator tapes (Temp-Tape 180°, Pharmacal).

Table 1. Facilities, primary function, number of rooms surveyed, and dirty-bedding exposure time for surveillance mice monitored for EHS prevalence in 2009

Facility	Primary function	No. of rooms	Exposure time (mo)
A	Barrier and experimental use	14	1.5–6
B	Semibarrier and experimental use	5	1–5.5
C	Breeding and experimental use	19	0.6–5
D	Breeding and experimental use	14	1.5–6
E	Experimental use	3	1.5–6.5
F	Experimental use	2	3–5.5

Surveillance mouse program. CD1 surveillance mice were bred in barrier facility A from stock periodically purchased from Charles River Laboratories (Andover, MA). Six surveillance mice were distributed at 6 wk of age to each mouse holding room with comprehensive evaluation by necropsy every 2 to 6 mo and cohort replacement every 6 mo. At the time of sampling feces for EHS PCR, each surveillance cage (10.5 × 19 × 6 in. static microisolation cage) held 2 to 6 mice. Surveillance mice were SPF for ecto- and endoparasites by fecal float, acetate tape test, and examination of fur plucks. In addition, mice were culture negative for specific murine pathogenic bacteria and serum antibody negative for all known exogenous murine viruses except murine norovirus, which was not routinely monitored.

Static microisolation cages were changed once weekly and ventilated cages were changed every other week, with as-needed spot changes. On a rotating basis, 30 colony cages receiving a full change were sampled by using a 1.35-oz. plastic scoop to transfer 1 aliquot of dirty bedding into a surveillance cage, ensuring a final ratio of 1 part clean to 2 parts dirty bedding. Surveillance mice were exposed weekly to dirty bedding from an average of 600 colony cages (approximately 1800 mice), with sampling systemically rotated among all cages in the holding room. This exposure protocol was demonstrated previously to efficiently transfer EHS between colony and surveillance mice with a concurrence as detected by PCR of 82% for Hh, 88% for Hr, and 94% for Hb.[32]

Sample collection for PCR detection of EHS. Fifty-seven holding rooms from 6 facilities were monitored for EHS by testing composite fecal samples from each cage of surveillance mice after dirty-bedding exposure times that ranged from 0.6 to 6.5 mo (Table 1). Cohort surveillance mice were placed in a clean cage until they collectively produced 10 fresh fecal pellets as a composite sample, which was stored at –80 °C until DNA extraction. Each set of surveillance mice was sampled once.

PCR assay of fecal samples. EHS infection status of surveillance mice was determined by PCR amplification of bacterial DNA extracted from composite fecal samples by using the QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA) according to manufacturer instructions. The protocol included the use of InhibitEX tablets (Qiagen) to absorb PCR inhibitors. In addition, each set of extractions included a positive control

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of PCR-negative *Helicobacter*-free feces spiked with 200 μL of culture medium (*Brucella* broth and 25% glycerol) containing approximately 10^9 cfu/mL of *H. pylori* SS1. A High Pure PCR Template Preparation Kit (Roche Diagnostics, Indianapolis, IN) and genus-level *Helicobacter* spp. primers (forward C05, reverse C97) were used to produce a 1.2-kb PCR product.[8] Genus-level PCR-positive and a small number of equivocal samples were assayed further by using species-specific primers for Hh,[24] Hb,[11] Hr,[11] and Ht,[6] with positive controls consisting of DNA extracted from pure cultures of these EHS. PCR products were electrophoresed through a 2% agarose gel, stained with ethidium bromide, and visualized with UV light. Surveillance mice were considered positive for *Helicobacter* infection if the composite fecal sample was positive by either genus-level or species-specific PCR.

16S rRNA gene sequencing. Samples positive by genus-level PCR that could not be discriminated at the species level by PCR were analyzed further by 16s rRNA gene sequencing. PCR products from *Helicobacter* spp. genus-level primers C05 and C97 were purified by using the QIAquick PCR Purification Kit (Qiagen) and sequenced by using the BigDye Terminator version 3.1 Cycle Sequencing Kit on an ABI 3500 genetic analyzer (Applied Biosystems, Foster City, CA).[7] Sequences were compared with the NCBI Genbank nucleotide database by performing a BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Comparison to previous prevalence data. In 1999, using PCR assay of cecal mucosal scrapings, we determined prevalence for Hh, Hr, and Hb in Tac:(SW)fBR sentinel mice used to monitor colony health status over 6 mo in 26 mouse holding rooms randomly selected from 4 separate mouse facilities.[32] The results confirmed sentinel mice acquired Hh, Hr, and Hb infection through our dirty bedding exposure protocol and that concurrence of infection status within surveillance cages and with colony mice were both 82% and higher, based on the type of EHS and exposure time to dirty bedding.

To determine whether the importation policy enacted in 2000 requiring all incoming mice to be *Helicobacter*-free correlated with a decrease in EHS prevalence, room histories were generated for those rooms surveyed during 1999 and 2009. Total mouse care days and the percentage of mice imported from EHS-free vendors were calculated for these rooms over this 10-y period. Although we had no practical means of tracking relocation of ET-rederived mice to specific rooms over this time period, data for campus-wide ET rederivation of mice either imported from other academic institutions or from existing facility colonies were evaluated.

Results

EHS prevalence. Genus-level PCR products were 1200 bp in length (Figure 1), and gel bands were strongly positive for 16 samples, or 28% of the 57 composite surveillance mouse fecal samples. Bands were faintly positive for 3 samples and negative for 38 samples. Species-specific PCR assays revealed expected gel band sizes[30] for Hh (417 bp), Hb (638 bp), Hr (166 bp), and Ht (122 bp; Figure 2). Hh was the most common EHS identified, with a prevalence of 26%, followed by Hb and Ht (both 16%) and Hr (12%). An additional EHS, Hm, was noted infrequently (4%); this helicobacter was identified by 16s rRNA sequencing of the genus-level PCR product and was detected in 2 sets of surveillance mice that had been in facility C for 89 d. At the time of sampling, surveillance mice had been exposed to dirty bedding from colony mice for 0.6 to 6.5 mo, however Hh, Hb, Hr, and Ht infections were acquired by surveillance mice within the first month of placement in the holding rooms.

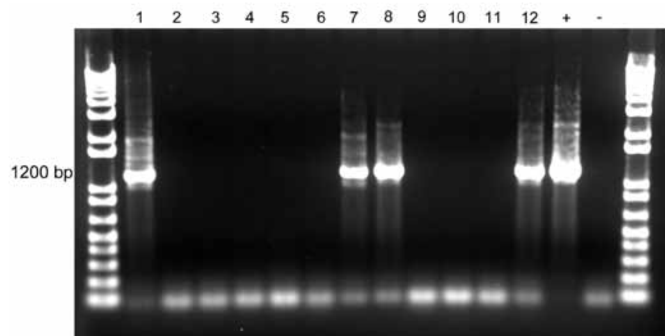

Figure 1. Representative EHS genus-level 1200-bp PCR product. The 2 outside lanes are 1-kb–plus ladders. Lanes marked 1 (positive for EHS) and 2 (negative PCR) represent composite fecal samples from surveillance mice in 2 holding rooms from facility D; lanes 3 through 12 (lanes 7, 8, and 12 positive for EHS PCR) represent 10 composite fecal samples from facility C. Lane 13 is positive-control DNA extracted from EHS-free feces spiked with *H. pylori* SS1; lane 14 is a negative control comprising of sterile water.

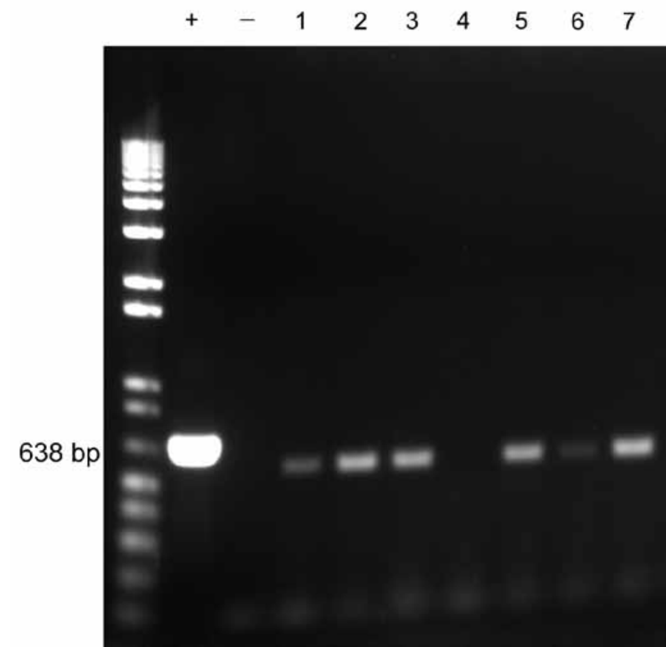

Figure 2. Representative species-specific 638-bp PCR product for *H. bilis* (Hb). The 1-kb–plus ladder is followed by positive-control DNA extracted from a pure Hb culture and then a negative control comprising of sterile water; lanes 1 through 5 represent composite fecal samples from surveillance mice in individual rooms located in facility D, and lanes 6 and 7 were samples from facility C. All samples expect lane 4 were PCR-positive for Hb.

The prevalence of EHS varied from none detected in facilities A, E, and F to 47% in facility C and 64% in facility D (Table 2); facilities C and D predominately housed long-term breeding colonies to generate experimental mice with lower importation of EHS-free mice. Facility B had one set of surveillance mice coinfected with Hb and Hr, yielding a prevalence of 20%, recognizing that only 5 sets of surveillance mice were sampled in facility B. In comparison to the other facilities, facilities E (3 rooms) and F (2 rooms) had smaller colonies of mice, originally obtained from the ET program or vendors and had minimal additional breeding for experimental mice. All surveillance mice sampled from facilities E and F were negative for EHS by PCR.

Multiplicity of EHS infection was detected in 74% of the PCR-positive composite fecal samples; therefore coinfections were

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Table 2. Prevalence of EHS infections expressed as the number of PCR-positive composite fecal samples from room surveillance mice

Infection status	Facility (no. of rooms surveyed)						Prevalence by species (%)
	A (14)	B (5)	C (19)	D (14)	E (3)	F (2)	
Genus level *Helicobacter*	0	1	7	8	0	0	28
H. hepaticus	0	0	7[b,c,e,g]	8[b,c,e,f,h]	0	0	26
H. bilis	0	1[a]	3[a,g]	5[d,f,h]	0	0	16
H. rodentium	0	1[a]	2[a,b,c,e]	4[b,c,e,h]	0	0	12
H. typhlonicus	0	0	4[e,g]	5[d,e,f,h]	0	0	16
H. mastomyrinus	0	0	2	0	0	0	4
Prevalence by facility (%)	0	20	47	64	0	0	not applicable

Infection status is represented via superscript as either one set of surveillance mice coinfected with [a]*H. bilis* and *H. rodentium*, [b]*H. hepaticus* and *H. rodentium*, [c]*H. hepaticus* and *H. bilis*, [d]*H. bilis* and *H. typhlonicus*, [e]*H. hepaticus*, *H. rodentium*, and *H. typhlonicus*, or [f]*H. hepaticus*, *H. bilis*, and *H. typhlonicus* or 2 sets of surveillance mice coinfected with [g]*H. hepaticus*, *H. bilis*, and *H. typhlonicus* or [h]*H. hepaticus*, *H. bilis*, *H. rodentium*, and *H. typhlonicus*.

more common than EHS monoinfections (Table 2). In facilities C and D, multiple EHS were detected by fecal PCR in 67% and 78% respectively, of surveillance mice. All 4 EHS (Hh, Hb, Hr, Ht) were detected in combinations of 2 to 4 species, without predominance of any one EHS. Monoinfections were limited to Hh and Hm; surveillance mice in one room in facility C and 2 rooms in Facility D were positive for Hh, and 2 other sets of facility C surveillance mice were PCR positive for Hm.

Comparison to historical data. Four of the 6 facilities (A, C, D, and E) surveyed in 2009 were also surveyed in 1999 (Table 3). Facilities B and F had not been constructed at the time of the 1999 survey. Hr prevalence went from 75% in facility A and 100% in facility E in the 1999 survey to being eradicated by 2009; this achievement was attributable to recognition of Hr infection by vendors in their production colonies and by restricting mouse importation to ET rederivation or EHS-free vendors. Hh prevalence in facilities B and C did not appreciably change over the 10-y period, and Hb actually increased in both facilities. Considering all facilities surveyed in 1999 and 2009, there was a substantial reduction in Hr prevalence from 76% to 12%, a moderate decrease in Hh from 41% to 26%, and a small increase in Hb prevalence from 6% to 16% of surveillance mice cohorts. In 1999, Ht had not been isolated and characterized, and therefore, surveillance mice were not monitored for Ht, preventing a comparison in prevalence of this organism in mice over the 10-y period.

The number of mice purchased from EHS-free vendors and the number of mouse care days for each of 9 holding rooms in 3 facilities surveyed in 1999 and 2009 were calculated over the 10-y period (data not shown) as a ratio to yield the relative percentage of mice imported as EHS-free (Table 4). Facility A, where EHS were eradicated by 2009, had 2 of the highest import percentages (0.05% and 0.2%). In comparison, rooms in facility C and D, where EHS remained endemic, had either a shift in the type of EHS (0.0% to 0.01% importation) or an increase (when importation was 0% or, in one case, 0.03%) or a decrease (0.01% importation) in the variety of EHS detected by PCR.

In total, 7745 mouse pups representing 531 unique genotypes were generated by ET rederivation from random-source mice and an additional 1863 mouse pups, representing 161 unique genotypes, were generated from ET rederivation of long-term breeding colonies within our institution.

Discussion

Analysis of PCR results from surveillance mice between 1999 and 2009 revealed that the greatest reduction in EHS prevalence in surveillance mice occurred in colonies importing the largest percentage of EHS-free mice from either vendors or ET rederivation. EHS remained endemic in colonies importing the fewest EHS-free mice. These results support our hypothesis that instituting a management policy requiring ET rederivation of random-source mice and the purchase of mice from vendor colonies screened for EHS infection status was responsible for decreased EHS prevalence on an institutional scale. A marked reduction in EHS infections was achieved without the intensive labor and expense associated with other techniques and without interference with investigators' experiments. In addition, the total number of *Helicobacter*-free mouse pups generated by ET rederivation and distributed globally to mouse facilities over the 10 y represented a large population that otherwise would have been imported from other random sources (academic institutions). These mice would have been likely to be infected with EHS and potentially other opportunist pathogens.[32]

The greatest reduction in EHS involved clearance of Hr infections from facilities A and E. Hr was first characterized in 1997[26] and was detected in 75% of surveillance mice from facility A and 100% of those from facility E in 1999. After the principal vendor used to provide mice for the facility A barrier eliminated Hr from its production colony and after we had segregated EHS-free ET and transgenic colonies from Hr-infected colonies, Hr was undetectable in facilities A and E in the 2009 survey. Reduction in Hh prevalence was less dramatic; a probable explanation is that Hh was most prevalent in facilities C and D, locations in which breeding typically was used to generate experimental mice, with infrequent importation of replacement mice. PCR results demonstrated that in the majority of facilities importing 0.03% or less of EHS-free mice between the first and second surveys, EHS were not eradicated, but an increase, decrease, or shift in the type of EHS occurred. The predominant epidemiologic trend for mice housed in these rooms included continued endemic inflections with Hh and Hr, acquisition of Hb infections, and newly identified Ht infections. PCR screening for Ht was not part of the 1999 survey, because this *Helicobacter* was not characterized until 2005.[6] Hb infections may have increased from breeding endemically infected mice to expand transgenic colonies for experiments.

PCR screening for EHS in surveillance mice documented infection (in decreasing prevalence) with Hh (26%), Hb (16%), Ht (16%), and Hr (12%). There was concurrence between

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Table 3. Percentages of surveillance mice PCR-positive for by assay of cecal scrapings in 1999 or composite fecal samples in 2009

	Facility A		Facility C		Facility D		Facility E		Facilities A, C, D, and E combined	
	1999	2009	1999	2009	1999	2009	1999	2009	1999	2009
H. hepaticus	0%	0%	40%	37%	71%	57%	0%	0%	41%	26%
H. bilis	0%	0%	0%	16%	14%	36%	0%	0%	6%	16%
H. rodentium	75%	0%	60%	11%	86%	29%	100%	0%	76%	10%

Table 4. Effect of importing EHS-free mice between 1999 and 2009

Facility	Room	EHS status[a]	% of mice imported from EHS-free vendors
A	1	Eradicated	0.05
	2	Eradicated	0.20
C	1	Eradicated	0.02
	2	Eradicated	0.07
	3	Shifted	0
	4	Increased	0.03
D	1	Decreased	0.01
	2	Shifted	0.01
	3	Increased	0

Facilities B, E and F were either not constructed or not sampled in 1999. The percentage of mice imported from EHS-free vendors was derived from the number of mice ordered from helicobacter-free vendors divided by the total number of mouse-care days for each room over the 10-y period.

[a]A status of 'eradicated' indicates that surveillance mice were PCR-negative for EHS. 'Shifted' indicates no change in number of *Helicobacter* spp. detected but that the species identified were different in the 2 surveys; 'increased' and 'decreased' indicate a change in the number of *Helicobacter* spp. detected.

genus- and species-specific PCR data in most samples and 100% agreement in samples positive for Hr infection. In 2 of 57 rooms, genus-level PCR was positive for EHS whereas the species-specific PCR assays for Hh, Hr, Hb, and Ht were negative. 16S rRNA sequence analysis of these samples identified both EHS as Hm. Genus-level PCR product bands obtained from surveillance mice in 3 rooms were equivocal on gels but were positive by species-specific assays. This pattern of findings likely reflects more efficient amplification of smaller PCR amplicons when EHS is present in low numbers.[35]

EHS typically were detected in composite fecal samples from surveillance mice within 1 mo of placement as sentinels. The exception was Hm, which was not detected until 89 d of dirty-bedding exposure; this apparent time delay may have been due to its low prevalence in colony mice. *H. mastomyrinus*, first identified in rodents in 2005,[27] was recently isolated in telomerase-deficient mice with chronic diarrhea and wasting.[5] The author also demonstrated that cecal contents inoculated into germfree Rag knockout mice receiving $CD4^+$ T cells from the telomerase-deficient mice resulted in severe granulomatous typhlocolitis, which also occurred in SPF Rag knockout and $IL10^{-/-}$ mice orally dosed with Hm.[5] However, to our knowledge, the current study is the first report of detecting Hm by PCR assay of feces from surveillance mice exposed to dirty bedding from colony mice. Although in previous assessments of surveillance mice, Hh was detected after 2 wk of biweekly exposure to dirty bedding,[10,19] Hr[32] and Hb[14] after 1 mo of either dirty-bedding or cage-contact exposure, and Ht after 2 mo of open-air exposure to adjacent infected cages,[1,12] the time of first detection of EHS is not likely comparable between studies, given the diversity of exposure protocols used in surveillance programs. However, the data do support the expectation that EHS in colony mice will be detected through dirty-bedding transfer to surveillance mice.

Although several studies have reported the presence of Hr and Ht in sex organs (testis, epididymis, ovary, uterus),[23,25] as well as of Hh in SCID fetuses,[18] indicating that vertical transmission of EHS can occur, most management techniques intended to contain or exclude EHS rely on preventing fecal–oral transmission. Personnel in all facilities contributing to the survey data reported here universally used sanitized forceps to transfer mice during cage change, microisolation cage filter tops, clean-to-dirty traffic flow, and a no-return policy. Because the EHS prevalence between 1999 and 2009 varied by facility, the results indicate only that the import requirement for EHS-free mice correlated with reduced EHS prevalence. Despite the conclusion that the importation policy made a key contribution, other epidemiologic factors warrant consideration. Genetic background, age, sex, reproductive status, immune competency, and research manipulation of colony mice may have affected the shedding of EHS to surveillance mice, but these variables could not be evaluated in the current study. Turnover in staff, diverse research protocols, and a rapidly expanding assortment of genetically modified strains of mice also may have altered EHS epidemiology. Lastly, the 1999 survey was conducted by using mucosal scrapings from the cecocolic junction of surveillance mice as samples for EHS-based PCR, whereas the 2009 survey used composite fecal samples. The mucus layer of the cecocolic junction is the preferred niche for EHS in mice,[33] and therefore the reliability of EHS detection by PCR of mucosal scrapings would not be affected adversely by unknown host factors, which might result in intermittent fecal shedding.[22] The difference in samples (tissue compared with feces) is likely not important, given that intermittent fecal shedding of EHS by colony mice would be compensated for by the large amount of dirty bedding transferred to surveillance mice over extended time periods.

As an essential component of the importation policy initiated in 2000, 3 vendors were identified that progressively eliminated EHS from their production barriers. The first vendor rederived their mouse production colonies in 2001, as confirmed by using genus-level PCR of feces and intestinal tissue. A second vendor has supplied *Helicobacter*-free mice since 2002, as determined by genus-level and species-specific PCR for Hb and Hh, with the exception of a *H. pullorum* outbreak in 2009.[2] The majority of a third vendor's barrier facilities were free of EHS by 2005, and all production areas were confirmed to be EHS-free by the end of 2006. Therefore, over the 10-y span between the 2 surveys of surveillance mice, vendors supplying mice to our colonies were progressively eradicating EHS. Therefore, as vendor colonies have been cleared of EHS, the importation policy is not restrictive to investigators ordering standard strains and stocks of mice.

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ET rederivation was another important component of the new importation policy. Over a 10-y period, the rederivation of imported mice from other academic institutions and resident mice infected with opportunistic murine pathogens resulted in the production of 9608 *Helicobacter*-free pups, representing 692 unique genotypes distributed throughout the campus. Facility A was the main production barrier for ET rederived and genetically modified mice; therefore EHS eradication was highly valued and achieved efficiently without labor-intensive cross-fostering or antibiotics. By eliminating traditional quarantine practices before release of random-source mice into established colonies, our importation policy very likely also reduced the risk of other opportunistic murine pathogens.

The current study is the first to demonstrate long-term success in decreasing the prevalence of *Helicobacter* infection by exclusive importation of EHS-free mice from vendors and by the use of ET rederivation for mice obtained from other academic institutions. This study also documents the persistent nature of EHS infection in mouse colonies and highlights the need for management practices that preclude introduction of EHS into mouse colonies.

Acknowledgments

We gratefully acknowledge Mr Steven Downey for assistance in establishing room and mouse purchase histories. Grant support includes T32 RR007036 (JGF), R01DK052413 (JGF), R01CA067529 (JGF), P01CA026731 (JGF), P30-ES02109 (JGF), and R01RR03237 (JGF).

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