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Enterohepatic *Helicobacter* Species Are Prevalent in Mice from Commercial and Academic Institutions in Asia, Europe, and North America▽

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The discovery of *Helicobacter hepaticus* and its role in hepatitis, hepatocellular carcinoma, typhlocolitis, and lower-bowel carcinoma in murine colonies was followed by the isolation and characterization of other *Helicobacter* spp. involved in enterohepatic disease. Colonization of mouse colonies with members of the family *Helicobacteriaceae* has become an increasing concern for the research community. From 2001 to 2005, shipments of selected gift mice from other institutions and mice received from specified commercial vendors were screened for *Helicobacter* spp. by culture of cecal tissue. The identities of the isolates were confirmed by genus-specific PCR, followed by species-specific PCR and restriction fragment length polymorphism analysis. Sequencing of the 16S rRNA gene was performed if the species identity was not apparent. The survey included 79 mice from 34 sources: 2 commercial sources and 16 research sources from the United States and 1 commercial source and 15 research sources from Canada, Europe, or Asia. *Helicobacter* spp. were cultured from the ceca of 62 of 79 mice. No *Helicobacter* spp. were found in mice from advertised *Helicobacter*-free production areas from two U.S. vendors. Multiple *Helicobacter* spp. were found in mice from one vendor's acknowledged *Helicobacter*-infected production area. The European commercial vendor had mice infected with novel *Helicobacter* sp. strain MIT 96-1001. Of the U.S. academic institutions, 6 of 16 (37%) had mice infected with *Helicobacter hepaticus*; but monoinfection with *H. bilis*, *H. mastomyrinus*, *H. rodentium*, and MIT 96-1001 was also encountered, as were mice infected simultaneously with two *Helicobacter* spp. Non-U.S. academic institutions had mice that were either monoinfected with *H. hepaticus*, monoinfected with seven other *Helicobacter* spp., or infected with a combination of *Helicobacter* spp. This survey indicates that 30 of 34 (88%) commercial and academic institutions in Canada, Europe, Asia, Australia, and the United States have mouse colonies infected with *Helicobacter* spp. Mice from 20 of the 34 institutions (59%) were most commonly colonized with *H. hepaticus* alone or in combination with other *Helicobacter* spp. These results indicate that a broad range of *Helicobacter* spp. infect mouse research colonies. The potential impact of these organisms on in vivo experiments continues to be an important issue for mice being used for biomedical research.

Naturally acquired *Helicobacter* infections have been reported in all commonly used laboratory rodent species (32). Several of the most frequently isolated species cause disease in selected strains of infected mice. *Helicobacter hepaticus* was first isolated from A/JCr mice in a long-term carcinogenesis study, in which the control animals developed a high incidence of hepatic tumors and hepatitis (5, 31). *H. hepaticus* is now known to cause hepatitis, liver tumors, cholesterol gallstones, inflammatory bowel disease, and colon cancer in susceptible strains of mice (2, 7, 16, 18, 32). The negative impact of natural infection with *H. hepaticus* was further documented in 12 National Toxicological Program studies (31). Nine of the 12 studies were confounded by *H. hepaticus*-induced hepatitis and hepatocellular carcinoma in control mice.

Other *Helicobacter* spp. are also known to cause disease in laboratory mice. Naturally acquired *H. typhlonius* causes typhlocolitis in immunocompromised mice (6, 11). *H. muridarum* may be associated with gastritis (22), whereas *H. bilis* is associated with moderate hepatitis in aged inbred and outbred mice (10, 12) and typhlocolitis and lower-bowel cancer in mice with intestinal barrier defects (17). Furthermore, *H. bilis* was reported to confound a study performed to determine the effect of chronic oral supplementation with creatinine in which both control and experimental outbred Swiss mice developed hepatitis (8). Maurer et al. (18) reported that C57L mice infected with *H. bilis* or coinfected with *H. hepaticus* and *H. rodentium* and fed a lithogenic diet developed cholesterol gallstones at an 80% prevalence by 8 weeks, whereas approximately 10% of the uninfected controls developed cholesterol gallstones. *H. rodentium* may also play a pathogenic role with other *Helicobacter* spp. and elicit diarrhea and typhlocolitis in immunocompromised mice (19, 28). In an experimental model, *Helicobacter* sp. strain MIT 96-1001 caused inflammatory bowel disease and cholangiohepatitis in SCID and immunocompetent A/J mice (29). Given the proven potential for *Helicobacter* spp. to confound research utilizing laboratory mice, we undertook a survey of mice from commercial vendors and academic re-

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TABLE 1. *Helicobacter* species-specific primer sets

Organism	Primer sequence (5′ to 3′)	Product size (bp)	Reference
H. typhlonius	Forward: AGGGACTCTTAAATATGCTCCTAGAGT Reverse: ATTCATCGTGTTTGAATGCGTCAA	122	3
H. rodentium	Forward: GTCCTTAGTTGCTAACTATT Reverse: AGATTTGCTCCATTTCACAA	166	27
H. bilis	Forward: AGAACTGCATTTGAAACTACTTT Reverse: GGTATTGCATCTCTTTGTATGT	638	10
H. hepaticus	Forward: GCATTTGAAACTGTTACTCTG Reverse: CTGTTTTCAAGCTCCCC	417	25

search facilities to determine the prevalence of *Helicobacter* spp. in mice used in biomedical research.

MATERIALS AND METHODS

Animals and tissue collection. A total of 79 mice were assessed for colonization with *Helicobacter* spp.. The majority of the mice (69/79) in this survey were genetically manipulated. They were sent to MIT principal investigators for research purposes from 16 research institutions in the United States and 16 research and commercial institutions in Canada, Europe, Australia, and Asia. In addition, 10 animals were purchased from two U.S. commercial vendors. The mice were euthanized with CO_2, and the cecum was collected from each mouse and stored in brucella broth containing 20% glycerol at −70°C until it was submitted for culture.

Histology. Representative sections of all the hepatic lobes, gallbladder, stomach, and ileocecal junction were fixed in 10% formalin, embedded in paraffin, and routinely stained with hematoxylin-eosin. A board-certified veterinary pathologist (P.N.) examined all tissue sections for lesions. Because, *Helicobacter* spp. typically cause inflammation within the stomach, liver, and ileocecocolic junction, special emphasis was placed on these organs. Briefly, the stomach, liver, and ileocecocolic junction were scored for inflammation or any other abnormal finding by using previously described criteria (2).

Bacterial isolation and characterization. Cecal tissue was homogenized in 1 ml of phosphate-buffered saline, and aliquots were placed on CVA (cefoperazone, vancomycin, and amphotericin B) plates or TVP (trimethoprim, vancomycin, and polymyxin B) plates and filtered through a 0.45-μm-pore-size filter onto Trypticase soy agar plates with 5% sheep blood (all from Remel Laboratories, Lenexus, KS). In-house-prepared selective medium plates were also used and contained the following: blood agar base (Oxoid; Remel), 5% horse blood (Quad Five, Ryegate, Montana), 50 μg amphotericin B/ml, 100 μg vancomycin/ml, 3.3 μg polymyxin B/ml, 200 μg bacitracin/ml, and 10.7 μg nalidixic acid/ml (all from Sigma Chemical Company, St. Louis, MO). After incubation under microaerobic conditions (culture vessels evacuated to 25 in. of mercury and filled with N_2-CO_2-H_2 at 80:10:10) at 37°C, suspect colonies were identified as *Helicobacter* on the basis of colony morphology, biochemical reaction (assessed for the enzymes catalase, oxidase, and urease), phase microscopy, Gram staining, and *Helicobacter* genus-specific PCR.

DNA extraction. For PCR of genomic DNA, isolates were grown on blood agar plates, harvested, and washed once with phosphate-buffered saline; and the High Pure PCR template preparation kit (Roche Molecular Biochemicals, Indianapolis, Indiana) was used for DNA extraction according to the manufacturer's specifications.

Genus-specific PCR. *Helicobacter* genus-specific primers that amplify a 1.2-kb product on the 16S rRNA gene were used as described previously (4).

RFLP and species-specific PCR. The 1.2-kb product from the genus-specific PCR was analyzed by restriction fragment length polymorphism (RFLP) analysis by the method previously published by Shen et al. (26). The resulting gel patterns were compared to the RFLP patterns of known *Helicobacter* spp. Those patterns resembling the patterns for *H. hepaticus*, *H. bilis*, *H. typhlonius*, and *H. rodentium* were subjected to species-specific PCR (Table 1) for species confirmation. The entire 16S rRNA gene from isolates that were negative by the species-specific PCR and isolates with RFLP patterns other than those for *H. hepaticus*, *H. bilis*, or *H. rodentium* was sequenced.

Sequence analysis. Amplification of the 16S rRNA cistrons, 16S rRNA gene sequencing, and analysis of the 16S rRNA data were performed as described elsewhere (21). For alignment, the 16S rRNA gene sequences were entered into RNA, a program designed for the analysis of 16S rRNA. The database contains more than 600 sequences for *Helicobacter*, *Wolinella*, *Arcobacter*, and *Campylobacter* strains and >2,000 sequences for other bacteria.

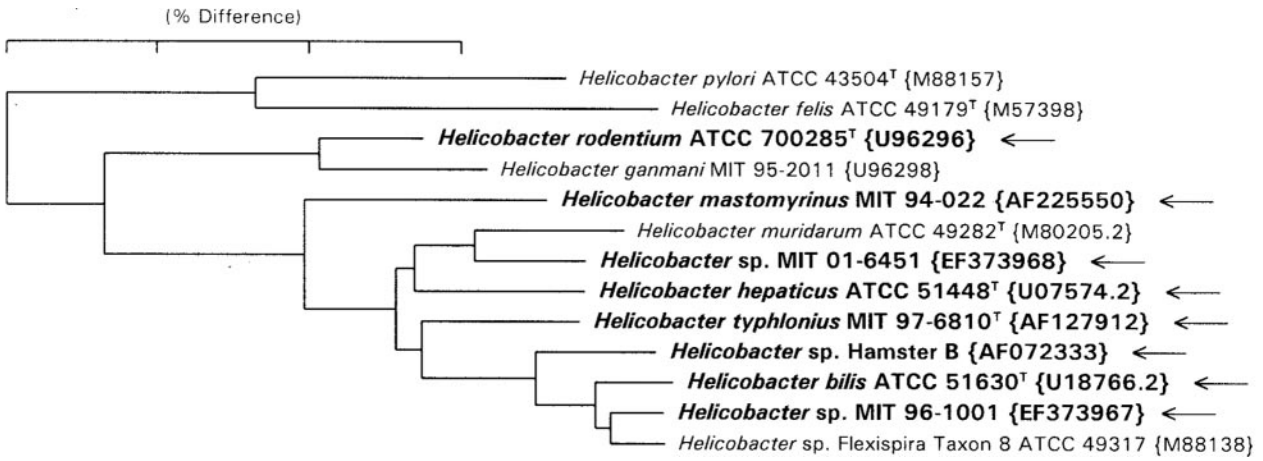

FIG. 1. Neighbor-joining phylogenetic tree of *Helicobacter* species recovered from mice. The GenBank accession number of each strain is given in braces. *H. pylori* and *H. felis* are included as an outgroup. The species identified in the current survey are shown in boldface and are marked with arrows.

TABLE 2. *Helicobacter* species isolated from commercial and research mouse colonies in Europe, Asia, Australia, and Canada[a]

Source	No. of mice tested/no. of *Helicobacter* spp. culture positive	WT/GEM: genotype (background)	Age (mo)	*Helicobacter* species identified	No. of isolates identified by:	
					PCR	Sequencing
Commercial company 1 (Switzerland)	2/2	GEM (B6/G9)	12+	*Helicobacter* sp. strain MIT 96-1001		2
Research institutions						
Institution 1 (Australia)	2/0	WT (C57BL/6)	13+	Negative	NA	NA
Institution 2 (Canada)	2/2	GEM: *Myr AKT*	9	*H. hepaticus*	2	NA
Institution 3 (Canada)	2/2	GEM: $Lnk^{-/-}$ (C57BL/6)	17	Mixed culture	NA	1
Institution 4 (Canada)	1/1	WT (C57BL/6)	8+	*H. hepaticus* mixed culture	1	
Institution 5 (England)	2/2	GEM: *R6/1* (CBA/C57BL/6 F_1)	6+	*H. hepaticus*	2	
Institution 6 (France)	1/0	GEM: *Apc Flox* (C57BL/6)	7+	Negative	NA	NA
Institution 7 (France)	2/1	GEM: *PO cre* (C57BL/6)	10+	*Helicobacter* sp. strain Hamster B (NCBI accession no. AF072333)		1
Institution 7 (France)	2/0	GEM: $IGF\text{-}IR^{-/+}$	9+	Negative	NA	NA
Institution 8 (Germany)	2/2	GEM: *D1 cre*	9+	*H. rodentium*	2	
Institution 9 (Japan)	2/2	GEM: $Lefty1^{+/-}$ (C57BL/6)	5+	*Helicobacter* sp. strain MIT 01-6451		1
Institution 10 (Japan)	2/2	GEM: WGA	7+	*Helicobacter* sp. strain MIT 01-6451		2
Institution 11 (The Netherlands)	2/2	GEM: *GFAP-cre* (FVB)	8	*H. hepaticus*	2	
Institution 11 (The Netherlands)	1/0	GEM: *p53-flox/Rb flox*	7+	Negative	NA	NA
Institution 12 (Netherlands)	1/1	GEM: $rad23B^{+/-}$ (C57BL/6)	12+	*H. hepaticus* and *H. typhlonius*	2	
Institution 13 (The Netherlands)	2/2	GEM: $cdx1^{-/-}$ (C57BL/6)	8+	*H. typhlonius*	2	
Institution 14 (Scotland)	3/3	GEM: *Alpha V Flox*	9	*H. hepaticus*	3	
Institution 14 (Scotland)	2/2	GEM: *Intavflox-cre*	20+	*H. mastomyrinus*		2
Institution 15 (Sweden)	2/2	GEM: *Cre 151*	6	*H. typhlonius*		1
Total	35/28				16	9

[a] WT, wild type; GEM, genetically engineered mice; NA, not applicable.

Nucleotide sequence accession numbers. The GenBank accession numbers for the strains examined in this study are included in Fig. 1.

RESULTS

Culture. Of 79 mice, 62 (78%) were positive by culture for *Helicobacter* spp. as assessed by biochemical reaction, phase microscopy, Gram staining, and *Helicobacter* genus-specific PCR. *Helicobacter*-infected mice came from 30 of the 34 institutions surveyed (Tables 2 and 3). Thus, the overall prevalence of *Helicobacter* spp. in mice from the institutions in this survey was 88%.

No *Helicobacter* spp. were found in mice from advertised *Helicobacter*-free production areas from two U.S. vendors. Multiple *Helicobacter* spp. were found in mice from one vendor's acknowledged *Helicobacter*-infected production area. The European vendor had mice culture positive for *Helicobacter* sp. strain MIT 96-1001. Shipments of mice from 15 of 16 U.S. research institutions (94%) had mice that were positive by culture for *Helicobacter* spp. Shipments from 13 of 15 non-U.S. research institutions (87%) had mice that were positive by culture for *Helicobacter* spp.

RFLP analysis followed by species-specific PCR. Of the 62 *Helicobacter*-positive cultures, 46 were assessed to be pure cultures. Digestion of the 1.2-kb PCR product with AluI and HhaI showed that 21 were *H. hepaticus*, 2 were *H. bilis*, and 3 were *H. rodentium*, according to their RFLP patterns. The identities of these 26 isolates were further confirmed by species-specific PCR. Fifteen of the remaining 20 isolates were submitted for sequence analysis of the 16S RNA gene (Tables 1 and 2). The remaining five isolates were not submitted for DNA sequence analysis because, based on their RFLP patterns and morphological characteristics, they were identical to an isolate obtained from a cage mate in the same shipment which was submitted for sequence analysis.

Analysis of mixed cultures. Pure cultures of individual species can be difficult to obtain due to the spreading nature of *Helicobacter* growth on agar. Mixed RFLP patterns and examination of bacteria by phase microscopy and Gram staining indicated that 16/62 (26%) cultures contained two or more *Helicobacter* spp. A pure culture of *H. hepaticus* and *H. typhlonius* from one set of coinfected mice was obtained only from U.S. institution 16. The remainder of the mixed cultures were

TABLE 3. *Helicobacter* species isolated from commercial and research mouse colonies in the United States[a]

Source	No. mice tested/no. of *Helicobacter* spp. culture positive	WT/GEM: genotype (background)	Age or characteristic	*Helicobacter* species identified	No. of isolates identified by:	
					PCR	Sequencing
Commercial						
Company 1	4/0	WT (AJ)	Various	Negative	NA	
Company 2	2/2	WT (BALB/c)	Retired breeders	*H. hepaticus*, *H. bilis*, and *H. rodentium*	6	
Company 2	2/2	WT (AJ)	Retired breeders	*H. hepaticus*, *H. bilis*, and *H. rodentium*	6	
Company 2	2/0	WT (AJ)	Retired breeders	Negative	NA	
Research institutions						
Institution 1 (California)	1/1	GEM: *HIF 1A* (129)	7 mo+	*H. hepaticus*	1	
Institution 2 (California)	1/1	GEM: *HYPOE* (C57BL/6)	11 mo+	*H. hepaticus*	1	
Institution 3 (Maryland)	2/0	GEM: c-*myc* (C57BL/6/CBA/J)	11 mo+	Negative	NA	
Institution 4 (Massachusetts)	2/2	GEM: $Smo^{-/-}$ (C57BL/6)	7 mo	*H. hepaticus*	2	
Institution 4 (Massachusetts)	2/2	GEM: *ICOS KO* (129)	15 wk	*H. bilis*	2	
Institution 5 (Massachusetts)	2/2	GEM: $Fibrogen^{-/-}$; $VWF^{-/-}$	11 mo	*H. hepaticus*	2	
Institution 6 (Massachusetts)	2/2	WT (C57BL/6)	7 mo+	*H. hepaticus*	2	
Institution 7 (Massachusetts)	2/2	GEM: *CARR*	8 mo+	*H. hepaticus*	2	
Institution 8 (Massachusetts)	2/2	GEM: *TLR2* (C57BL/6)	9 wk	*H. mastomyrinus*		1
Institution 8 (Massachusetts)	2/2	WT (B6/129)	6 wk	*H. mastomyrinus*		1
Institution 8 (Massachusetts)	1/1	GEM: *INS-GASGgly* (FVB)		*H rodentium*	1	
Institution 8 (Massachusetts)	1/0	GEM: *TFF2* (C57BL/6)	7 mo+	Negative	NA	
Institution 8 (Massachusetts)	1/0	GEM: *INS-GAS* (C57BL/6)		Negative	NA	
Institution 9 (New York)	1/1	GEM: *DYT* (C57BL/6)	9 mo+	*Helicobacter* sp. MIT 96-1001		1
Institution 9 (New York)	1/1	GEM: *DYT* (C57BL/6)	9 mo+	*H. hepaticus* and *H. typhlonius*	2	
Institution 10 (New York)	2/2	WT (FVB)	11 mo+	*H. hepaticus* and *H. typhlonius*	4	
Institution 11 (New York)	1/1	GEM: $E2F1^{+/-}$	7 mo+	*H. hepaticus* and *H. typhlonius*	2	
Institution 12 (Ohio)	2/2	GEM (C57BL/6-AJ)	8 mo+	*H. hepaticus*	2	
Institution 13 (Ohio)	2/2	GEM: *Pdx-cre*	12 mo+	*H. hepaticus* and *H. typhlonius*	4	
Institution 14 (Tennessee)	2/2	GEM: *p48-cre*	10 mo+	*H. hepaticus* and *H. typhlonius*	4	
Institution 15 (Texas)	1/1	GEM: *mdm2* (129)	7 mo+	*H. hepaticus* and *H. typhlonius*	2	
Institution16 (Texas)	1/1	GEM: *2-MHCGSK-3BS9A*	12 mo+	*H. hepaticus* and *H. typhlonius*	2	1
Total	44/34				47	4

[a] WT, wild type; GEM, genetically engineered mice; NA, not applicable.

assessed by using the species-specific primers listed in Table 1. Figure 2 shows the results of the species-specific PCR for the mixed cultures and for other known murine *Helicobacter* spp. One shipment from a U.S. commercial vendor had mice that were positive by PCR with primers specific for *H. hepaticus*, *H. bilis*, and *H. rodentium*. Six U.S. research institutions and one European research institution had mice that were positive by PCR with primers specific for *H. hepaticus* and *H. typhlonius*. Only *H. hepaticus* could be identified with species-specific primers and DNA from a mixed culture from mice from institution 4 (Canada). The mixed *Helicobacter* spp. obtained from institution 3 (Canada) could not be identified with species-specific primers.

Sequencing of pure cultures. Of the 20 isolates not identified with the species-specific primers, 14 isolates were analyzed by complete sequencing of the 16S rRNA gene (Tables 2 and 3). Sequence analysis identified *H. mastomyrinus* (four isolates), *H. typhlonius* (two isolates), MIT 96-1001 (three isolates), Hamster B (NCBI accession no. AF072333) (one isolate), and novel *Helicobacter* species strain MIT 01-6451 (three isolates). One DNA sample from a culture from mice from institution 3 (Canada) could not be identified because it contained DNA from more than one organism. Figure 1 shows a phylogenetic tree of the *Helicobacter* species recovered from the mice. The tree includes *H. pylori* and *H. felis* as outgroups. The *Helicobacter* species recovered from the mice examined in this study are shown in boldface.

Distribution of *Helicobacter* species. Overall, this survey found that 2/34 institutions (6%) had mice colonized with at least three *Helicobacter* spp., 10/34 (29%) had mice colonized with two *Helicobacter* spp., and 15/34 (47%) had mice colonized with a single *Helicobacter* species. Institutions in the United States were more likely to have mice colonized with two or more *Helicobacter* spp., including *H. hepaticus* with *H. rodentium* and *H. bilis* (1/3 commercial institutions), MIT 96-1001 with *H. hepaticus* and *H. typhlonius* (institution 9), and *H. mastomyrinus* with *H. rodentium* (institution 8). *H. hepaticus* and *H. typhlonius* were identified in mice from 7 of 16 research institutions. Outside the United States, one research institution had mice colonized with *H. mastomyrinus* and *H. hepaticus* (institution 14) and another had mice colonized with *H. hepaticus* with *H. typhlonius* (institution 12).

Among the mice from the 34 institutions surveyed, *H. hepaticus*, alone or in combination with other *Helicobacter* spp., was the organism most predominantly found (59%), followed by *H. typhlonius* (26%) and MIT 96-1001, *H. rodentium*, and *H.*

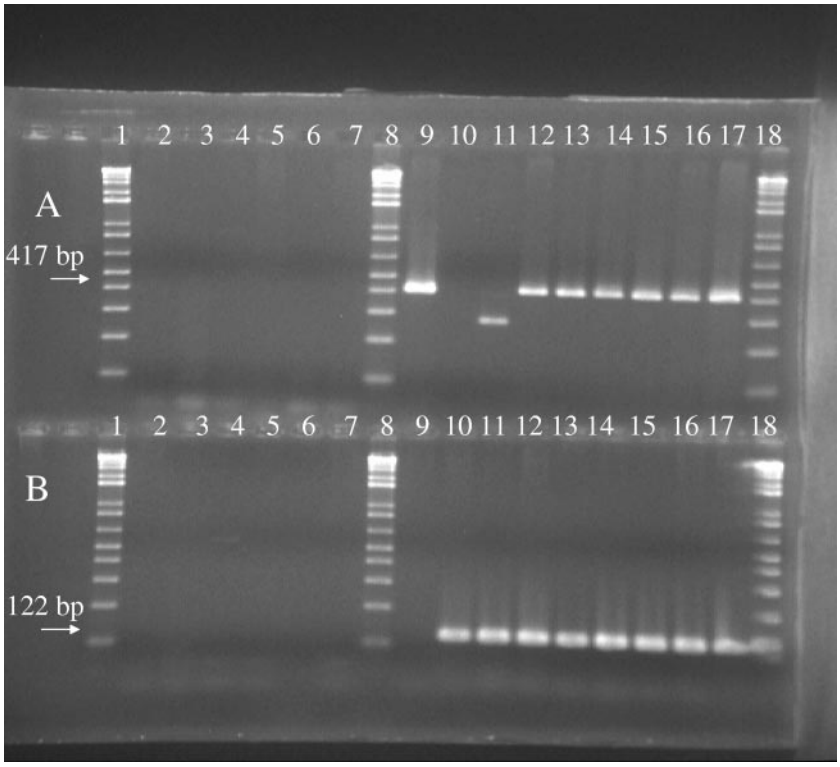

FIG. 2. Results of species-specific PCR for (A) *H. hepaticus* and (B) *H. typhlonius* in mixed cultures. Lanes: 1, 1-kb plus ladder; 2, Hamster B (NCBI accession no. AF072333); 3, *H. bilis*^{T}; 4, *H. mastomyrinus*^{T}; 5, MIT-1001; 6, *H. rodentium*^{T}; 7, novel MIT 01-6451; 8, ladder; 9, *H. hepaticus*^{T}; 10, *H. typhlonius*^{T}; 11, *H. typhlonius* (U.S. institution 16); 12 to 17, mixed cultures from European institution 12 and U.S. institutions 9 to 11 and 13 to 15, respectively; 18, ladder. PCR of these DNA samples with primers specific for *H. bilis* and *H. rodentium* gave negative results.

mastomyrinus (6% each). One novel *Helicobacter* species was also cultured from mice from two Asian institutions. Mice in the United States were more likely to be colonized with *H. hepaticus.* Fifteen of the 18 institutions had mouse colonies infected with *H. hepaticus* alone or with other species (90%). The prevalence of *H. typhlonius* in mice from the 18 institutions was also high in the United States (39%). Mice from the 16 non-U.S. institutions were the most likely to be colonized with *H. hepticus* alone or with other species (37%). However, mice from non-U.S. institutions were also monocolonized with several other *Helicobacter* spp.

Mice obtained from 11 European institutions were monocolonized with diverse *Helicobacter* spp., including *H. hepaticus*, *H. typhlonius*, MIT 96-1001, *H. rodentium*, and *H. mastomyrinus*. Mice from both Asian institutions were colonized with the same novel *Helicobacter* sp. (strain MIT 01-6451). Shipments from three Canadian institutions had mice that were colonized with *H. hepaticus* or were cocolonized with more than one *Helicobacter* species. Mouse colonies in the United States were colonized with six different species (including *H. hepaticus*, *H. typhlonius*, MIT 96-1001, *H. rodentium*, *H. mastomyrinus*, and *H. bilis). H. ganmani* was not found in mice from any of the institutions screened. *H. bilis* was not found in mice from the European institutions.

Histopathology. No pathology consistent with *Helicobacter* infection or the age of the mouse was noted in any of the mice. Of those mice whose background strain could be determined, most were of the C57BL/6 background, a strain of mouse known to be resistant to the pathology caused by enterohepatic *Helicobacter* spp. (16, 31).

DISCUSSION

This study found an 88% prevalence of *Helicobacter* spp. in mouse colonies from commercial and research institutions around the world. The finding that *H. hepaticus* is the predominant species in the United States confirms the findings of Shames et al. (25), who also used bacterial culture and PCR to identify *H. hepaticus* colonization in murine colonies. In a 1996 survey of major commercial vendors in the United States, 28 different strains from a total of 160 mice from four major U.S. vendors were surveyed by bacterial culture. All mice from 2 outbred strains from one vendor were colonized with *H. hepaticus*, and 9 of 13 inbred mouse strains from another vendor were also infected with *H. hepaticus* (25). In 1998, Riley et al. (23) used fecal PCR and RFLP analysis to identify *H. hepaticus*, *H. muridarum*, and *H. bilis*, the most common rodent helicobacters known at that time. They reported that of 508 mice, 10.4% were colonized with *H. hepaticus*, 17.1% were colonized with *H. bilis*, and <1% had *H. muridarum* colonization. Our laboratory has reported that one vendor had mice colonized with *H. bilis*, and a U.S. research institution that also had *H. bilis* in its colony obtained mice from that vendor (8). Thus, the distribution of *Helicobacter* species in the research population is, not surprisingly, related to the source of the mice.

In Japan, Goto et al. (13) surveyed 820 mice from 47 colonies in universities, breeding companies, pharmaceutical companies, and national research institutions obtained in 1997 and 1998. Using fecal reverse transcription-PCR, they found that the following strains were present in the colonies: *H. hepaticus* (25.5% of the colonies); *H. rodentium* (38.3%); *H. hepaticus* and *H. rodentium* (5.7% each); and *H. typhlonius*-like, *H. bilis*, and *H. westmeadii*-like (2.1% each). *H. westmeadii* was later identified as *H. cinaedi* (30). A novel *Helicobacter* was found in both of the two institutions in Japan that we surveyed. Thus, other *Helicobacter* species are present in Japanese institutions, in addition to those that Goto et al. (13) reported in their paper.

Using fecal PCR-denaturing gradient gel electrophoresis, Grehan et al. surveyed eight mice from three suppliers in Australia (14). They reported that four of eight mice were colonized with *H. ganmani*, two of eight had *H. bilis*, three of eight had *H. hepaticus*, and one of eight had two unique isolates, in addition to *H. ganmani*. One mouse was negative for *Helicobacter* DNA. The research institution that we surveyed in Australia had mice that were negative by culture for all helicobacters. We did not use anaerobic conditions for culture and did not isolate *H. ganmani* from any mice. While it has been reported that *H. ganmani* grows only anaerobically (24), we have found that the strain obtained from CCUG grows both anaerobically and microaerobically in our laboratory. This may be due to our use of culture in jars evacuated to 25 in. of mercury and then filled with an 80:10:10 mixture of nitrogen-carbon dioxide-hydrogen rather than anaerobic jars with gas-generating packs, as described by Robertson et al. (24). Also, the *Helicobacter* species present in these mice and the number of mice infected may be underestimated since PCR of DNA directly isolated from fecal samples is more sensitive than culture (9, 20, 25). However, the use of culture techniques allowed the characterization of the *Helicobacter* spp. isolated and the identification of novel *Helicobacter* spp.

Nilsson et al. (20) also used PCR-denaturing gradient gel electrophoresis but added pyrosequencing to detect *Helicobacter* spp. in 15- to 26-week-old mice from four different animal facilities in Sweden. One animal facility had mice negative for *Helicobacter*. The other vivaria had mice infected with multiple *Helicobacter* spp., including *H. bilis*, *H. hepaticus*, *H. typhlonius*, *H. ganmani*, and *H. rodentium*. The one research institution that we surveyed in Sweden had mice infected with *H. typhlonius*.

In Germany, Bohr et al. (1) reported on the prevalence and spread of enterohepatic *Helicobacter* spp. in mice from a specific-pathogen-free animal facility. Eighty-five percent of the mice were colonized, some with multiple *Helicobacter* species. Five different species were identified by PCR: *H. ganmani*, *H. hepaticus*, *H. typhlonius*, and isolates resembling Hamster B and MIT-5357. The one German institution in this study had mice colonized with *H. rodentium*.

Other countries in this study that have not previously published information on the prevalence of helicobacters in murine research colonies included Switzerland, England, The Netherlands, Scotland, France, and Canada. All were found to have colonies infected with *Helicobacter* spp.

The lack of pathology consistent with *Helicobacter* infection in this study was expected, since most mice in this study were of a C57BL/6 background, and such mice are known to be resistant to enteric and hepatic diseases (16, 31). Nilsson et al. (20) reported that the only pathology seen in their study of 42 young adult mice was hepatitis associated with *H. hepaticus*-monoinfected C57BL/6 *ApoE*$^{-/-}$ mice and BALB/cA mice infected with multiple helicobacters (*H. hepaticus*, *H. bilis*, *H. rodentium*, and *H. typhlonius*). Goto et al. (13) surveyed 820 mice 9 weeks of age or older and found that the only pathology observed was in 5/174 (2.8%) mice colonized with *H. rodentium* and *H. rodentium*-like strains. They reported multiple white foci on the livers and suggested that *H. rodentium* may be associated with hepatitis.

In summary, more than 10 years have elapsed since the original isolation of *H. hepaticus*, and even though it may confound the results of experiments with infected mice, this *Helicobacter* is still prevalent in mouse colonies throughout the world. In addition, other *Helicobacter* spp. known to cause disease in mice are also widespread (8–11, 13–15). Several vendors do maintain mice in *Helicobacter*-free breeding areas. Requesting animals from these areas, importing them into *Helicobacter*-free rooms, and implementing strict animal husbandry procedures are ways to control infection. For existing lines of mice, rederivation and housing under *Helicobacter*-free conditions remain the most reliable options (32).

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