

## Identification of Murine Helicobacters by PCR and Restriction Enzyme Analyses

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Three murine helicobacter species have recently been identified: *Helicobacter hepaticus*, *Helicobacter muridarum*, and *Helicobacter bilis*. Infections with *H. hepaticus* and *H. bilis* have been associated with hepatitis and hepatic neoplasia. In this study, oligonucleotide primers were designed from regions of the 16S rRNA gene that are conserved among members of the *Helicobacter* genus. The assay amplified the expected 374-bp product from all three rodent *Helicobacter* species and was able to detect as little as 5 pg of *H. hepaticus*, *H. bilis*, or *H. muridarum* DNA. The specificity of the reaction was determined by testing cecal DNA from uninfected mice and mice with documented *Helicobacter* infections and by testing DNA from other bacterial genera. A product of the expected size was generated with cecal DNA from *Helicobacter*-infected mice but not with DNA from uninfected mice. With the exception of that of "*Flexispira rappini*," which is closely related to the *Helicobacter* genus, DNA from other bacterial genera was not amplified with the *Helicobacter* genus-specific primers. *Mbo*I, *Mae*I, and *Hha*I restriction enzyme analyses of the amplified product were able to differentiate among the murine *Helicobacter* species but could not differentiate *H. bilis* from "*F. rappini*." To distinguish *H. bilis*, a reverse primer based on *H. bilis* 16S rRNA sequence was designed. PCR with the *H. bilis*-specific reverse primer (Hbr) and the *Helicobacter* genus-specific forward primer (H276f) amplified *H. bilis* DNA but not DNA from "*F. rappini*" or other rodent helicobacters. Examination of a large number of murine cecal tissues with this combination of PCR assays and restriction enzyme analyses indicated that *H. hepaticus* and *H. bilis* infections are widespread in laboratory mouse and rat colonies.

In recent years, members of the genus *Helicobacter* have received increasing attention. *Helicobacter pylori* has been implicated as the causative agent of gastritis and peptic ulcers in humans (7, 8). For rodents, three murine helicobacter species have been identified: *Helicobacter hepaticus*, *Helicobacter muridarum*, and *Helicobacter bilis*. *H. hepaticus* was initially reported to be present in mice used in a long-term toxicology study (15, 17). Both experimental and untreated control mice presented with chronic active hepatitis and a high incidence of hepatic tumors, which invalidated the results of the toxicologic study. Subsequent investigations revealed a helical bacterium, *H. hepaticus*, at the periphery of lesions. The organism was later identified to be present in the lower gastrointestinal tracts of mice (4) and has also been associated with inflammatory bowel disease (16). *H. bilis*, a fusiform bacterium, was initially identified as occurring in the bile, livers, and intestines of aged mice exhibiting multifocal chronic hepatitis (5). *H. muridarum* colonizes the lower bowels of mice and rats and has been found in the gastric pits of the stomachs of BALB/c mice (6, 10, 11).

The association of these helicobacter organisms with hepatitis and hepatic neoplasia has raised great concern in the biomedical community about the effects these agents may have on research investigations that utilize infected mice and rats (3, 15, 16). The consequences of infection on the host animal have not been thoroughly characterized, but preliminary studies have shown changes in serum enzymes and bile acids in infected mice (15, 17). Thus, infections of laboratory mice and rats with these agents may alter results of research investiga-

tions. These concerns are further complicated by the insidious nature of infections with these agents. Because infections are usually subclinical, investigators may not be aware that their laboratory rodents are infected. To limit the potential deleterious effects of these bacteria on research investigations, infected rodents must be identified. Culture of helicobacter organisms can be used to identify infected animals; however, the bacteria are fastidious and initial in vitro isolation requires special microaerophilic conditions and 5 to 7 days for bacterial growth (4, 5). Detection of helicobacters by culture is further complicated by the presence of normal flora, since the preferred site for isolation of the bacteria is the gastrointestinal tract. Because of the drawbacks of culture, a rapid, specific alternative method to detect *Helicobacter*-infected laboratory rodents would be of great value to the research community.

In this study, we report development of a molecular assay that identifies helicobacter-infected rodents. The assay utilizes PCR amplification with primers designed from regions of the 16S rRNA gene that are conserved among members of the *Helicobacter* genus. Samples yielding a product of the expected size are subjected to restriction enzyme analyses or a species-specific PCR assay to identify to species level the helicobacter organism involved in the infection. Evaluation of laboratory mice and rats with the developed PCR assay indicated that infections with *H. hepaticus* and *H. bilis* are widespread and that *H. muridarum* is more commonly found in rats than in mice.

### MATERIALS AND METHODS

**Bacterial strains and cultivation.** An *H. hepaticus* isolate was obtained from an adult male A/JNc mouse from a colony in which infection was endemic (15, 17). The liver was removed aseptically, minced, and inoculated onto brucella blood agar plates (Remel Laboratories, Lenexa, Kans.). Plates were incubated for 5 to

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TABLE 1. Oligonucleotide primers used to amplify the 16S rRNA gene sequences of helicobacters

Primer	Sequence (5' to 3')	Positions <sup>a</sup>
Broad-range prokaryotic		
g2	GCTTAACACATGCAAGTCGAA	46-68
cg4	GGTGAGTACCAGGTATCTAATCC	810-785
p93E	CCGCACAAGCGGTGGAGCA	930-950
p13B	AGGCCCGGGAACCTATTCC	1390-1371
Helicobacter sequencing		
H419r	AATCCTAAAACCTTCATCCTC	419-406
H593f	TAAGTCAGATGTGAAATCC	593-611
<i>Helicobacter</i> genus specific		
H276f	CTATGACGGGTATCCGGC	276-293
H676r	ATTCCACCTACCTCTCCCA	676-658
<i>H. bilis</i> -specific reverse, Hbr	TCTCCCATACTCTAGAAAAGT	664-644

<sup>a</sup> The positions within the *E. coli* 16S rRNA gene sequence that correspond to the 5' and 3' ends of each primer are shown. Approximate positions are given for *Helicobacter*-specific primers.

7 days at 37°C under microaerophilic conditions in vented jars containing N<sub>2</sub>, H<sub>2</sub>, and CO<sub>2</sub> (90:5:5). Type strains of *H. bilis* (ATCC 51630), *H. muridarum* (ATCC 49282), and "*Flexispira rappini*" (ATCC 43879) were obtained from the American Type Culture Collection (Rockville, Md.) and cultured on 5% blood agar plates as described above.

**Histopathology.** Mouse liver tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at a thickness of 5 µm, and stained with either hematoxylin and eosin or Steiner's silver stain. Stained sections were examined microscopically for lesions and bacteria consistent with those described for rodent helicobacter infections.

**Isolation of DNA.** To isolate DNA from cultured organisms, bacteria were harvested from agar plates by resuspending the organisms in phosphate-buffered saline, pH 7.4 (PBS). Suspensions were centrifuged at 12,000 × g, and the bacterial pellet was washed with PBS. DNA was extracted by using a QiAmp Tissue Kit (Qiagen Inc., Chatsworth, Calif.) according to the manufacturer's instructions. DNA content and purity were determined spectrophotometrically by measuring the A<sub>260</sub>/A<sub>280</sub> optical density ratio. To isolate DNA from liver and cecum tissue samples, tissues were removed aseptically from euthanized mice and frozen at -20°C. A 25-mg section of frozen liver or cecum was processed for DNA isolation by using the QiAmp Tissue Kit described above.

**Oligonucleotide primers.** Oligonucleotide primers (Table 1) were synthesized at the DNA Core Facility, University of Missouri, Columbia. The sequences of broad-range prokaryotic primers (g2, cg4, p13B, and p93E) were adapted from previous studies (2, 12). *Helicobacter*-specific sequences were selected on the basis of alignments performed with the EuGene software package (Baylor College of Medicine, Houston, Tex.). All sequence data were obtained from GenBank, with the exception of those for *H. hepaticus* and *H. bilis*, which were obtained in our laboratories.

**DNA sequencing.** Broad-range prokaryotic primers (g2 and p13B) were used in PCRs to amplify a 1,344-bp fragment from the 16S rRNA genes from *H. hepaticus* and *H. bilis* (Table 1). PCR products were purified on 3.5% polyacrylamide gels. The nucleotide sequences of the amplified products were determined by the *Taq* dideoxy-chain termination method with a commercially available kit (*Taq* Dye Deoxy Terminator Cycle Sequencing Kit; Applied Biosystems, Inc., Foster City, Calif.) by using additional broad-range prokaryotic (cg4 and p93E) and helicobacter (H593f and H419f) sequencing primers (Table 1), which were designed on the basis of sequence data obtained with broad-range prokaryotic primers. Sequence data were analyzed with the EuGene software package.

**PCR amplification.** All reactions were performed in a 50-µl volume with an automated Perkin-Elmer 9600 thermocycler. Reaction mixtures contained each oligonucleotide primer at 1 µM, PCR buffer (10 mM Tris-HCl, 1.5 mM MgCl<sub>2</sub>, 50 mM KCl [pH 8.3]), 3.0 U of *Taq* polymerase, and 1.25 µg of template DNA, unless otherwise stated. Samples were heated at 94°C for 5 min and then subjected to 35 cycles consisting of denaturation (94°C, 2 s), primer annealing (53°C, 2 s), and extension (72°C, 30 s). PCR products (16 µl) were electrophoretically separated in a 2% NuSieve agarose gel (FMC BioProducts, Rockland, Maine), stained with ethidium bromide, and visualized under UV light. DNA markers of known sizes were run on each gel to determine reaction product sizes.

To test the sensitivity of the assay, 10-fold serial dilutions of *H. hepaticus* and *H. muridarum* DNA, ranging from 100 ng to 100 fg, were used as the template in the helicobacter PCR assay with genus-specific primers. To simulate diagnostic conditions, assays were performed in the presence of 1.25 µg of DNA extracted from the cecum of a mouse which was negative for helicobacters by culture of

intestine and fecal specimens and by histological evaluation of the liver with Steiner's stain. To test the specificity of the assay, DNA preparations isolated from *Streptococcus faecalis*, *Escherichia coli*, *Proteus mirabilis*, *Bacillus subtilis*, *Campylobacter jejuni*, and "*F. rappini*" were used in PCR assays with the *Helicobacter* genus-specific primers.

**Restriction enzyme analysis.** To identify the helicobacter organisms to species level, PCR products were digested in separate reactions with either *Mbo*I, *Mae*I, or *Hha*I restriction endonuclease. Briefly, 9 µl of amplified PCR product was digested for 1 h with 2 U of the appropriate restriction endonuclease under conditions recommended by the enzyme's manufacturer. Restriction products were separated by electrophoresis and visualized as described above.

**Animal experiments.** Ten A/JNcR and 10 BALB/c male mice, 6 to 19 months of age, were obtained from colonies infected with *H. hepaticus* and *H. bilis*, respectively. The infectious status of these colonies was confirmed by culture of the helicobacter organisms and histopathological observation of bacteria that were consistent in size and morphology with *Helicobacter* organisms in liver tissues. Liver and intestinal samples were aseptically collected from each euthanized mouse and stored at -20°C until used. DNA was isolated from tissues and evaluated by PCR as described above. Mice of unknown infection status submitted for diagnostic evaluation to the University of Missouri Research Animal Diagnostic and Investigative Laboratory (Columbia, Mo.) were treated in an identical manner. All animal procedures were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (9).

## RESULTS

**Sequences of the 16S rRNA genes.** The nucleotide sequences for the 16S rRNA genes of *H. hepaticus* and *H. bilis* were determined in our laboratories since they were not available at the time these studies were initiated. For *H. hepaticus*, purified DNA was prepared from *H. hepaticus* cultured from an A/JNcR mouse as described above. For *H. bilis*, DNA was purified from the liver of an *H. bilis*-infected BALB/c mouse obtained from an endemically infected colony (5). DNA preparations were amplified with broad-range prokaryotic primers for the 16S rRNA gene, and approximately 1,300 bp of the amplified product was sequenced. Comparison of the determined sequences with that subsequently published by Fox and colleagues (4, 5) revealed sequence homologies of greater than 99.7% identity, confirming the identities of the *H. hepaticus* and *H. bilis* isolates used in this study.

**Evaluation of *Helicobacter* PCR primers.** The nucleotide sequences of the 16S rRNA genes from *H. hepaticus*, *H. bilis*, *H. muridarum*, *Helicobacter mustelae*, *Helicobacter felis*, and *H. pylori* were compared, and genus-specific primers were designed from conserved regions (Table 2). Specificity of the genus-specific primer set was determined by amplifying DNAs prepared from in vitro-cultured organisms. The expected 374-bp DNA fragment was amplified from DNAs from all members of the *Helicobacter* genus tested (Fig. 1). Template DNAs from other bacterial genera commonly found in the

TABLE 2. Comparison of genus-specific primer sequences with the DNA sequences of various organisms

Primer(s) or organism	Sequence (5' to 3')	
	Forward	Reverse
H276f and H676r <sup>a</sup>	CTATGACGGGTATCCGGC	ATTCCACCTACCTCTCCCA
<i>H. hepaticus</i>	----- <sup>b</sup>	-----
<i>H. bilis</i>	-----	-----
<i>H. muridarum</i>	-----	-----
<i>H. felis</i>	-----	-----
<i>H. pylori</i>	-----	-----
<i>E. coli</i>	-G-C--TCCC--G-T--T	---T---CC----A-G-
<i>C. jejuni</i>	-----CT--A-T--T	--N---T--G-----T
<i>P. mirabilis</i>	GGAT-AG-TA--GGT-A	G--T-AGA-G-AGT-----

<sup>a</sup> H276f, forward primer; H676r, reverse primer.

<sup>b</sup> Dashes indicate sequence homology with the H276f primer (forward) or H676r primer (reverse).

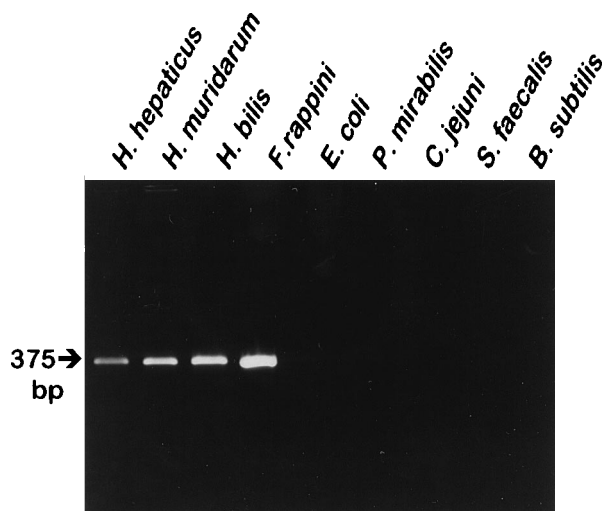


FIG. 1. Ethidium bromide-stained agarose gel showing products resulting from amplification of DNA from various bacteria.

mouse intestine were also evaluated for PCR amplification with the *Helicobacter* genus-specific primers. A product of the expected size was not observed with DNA from *S. faecalis*, *E. coli*, *P. mirabilis*, and *B. subtilis*. Specificity of the assay was further examined by evaluating amplification of two additional bacterial species that are infrequently found in the intestinal tracts of mice, *C. jejuni* and "*F. rappini*." Results indicated that "*F. rappini*" DNA was amplified, but *C. jejuni* DNA was not.

The sensitivity of the PCR assay was determined by amplifying 10-fold serial dilutions of either *H. hepaticus* or *H. muridarum* DNA. To simulate diagnostic conditions, all reactions were carried out in the presence of 1.25  $\mu$ g of DNA isolated from the cecum of an uninfected mouse. An amplification product of the expected size was detected in ethidium bromide-stained gels when as little as 5 pg of DNA from either *H. hepaticus* or *H. muridarum* was used as the template (Fig. 2).

**Identification of rodent helicobacters to species level.** Restriction enzyme analysis was examined as a method to differentiate rodent helicobacters from one another. Computer analysis of the DNA sequence which is expected to be amplified with the H276f and H676f primers revealed a unique pattern of restriction enzyme cleavage sites for each of the three murine helicobacter organisms with the enzymes *Mbo*I, *Mae*I, and *Hha*I (Fig. 3). To confirm that restriction enzyme analyses could be used to differentiate these bacteria, PCR products obtained with the genus-specific primers and template DNA from *H. hepaticus*, *H. muridarum*, and *H. bilis* were digested with *Mbo*I, *Hha*I, and *Mae*I in separate reactions and the restriction products were evaluated by agarose gel electrophoresis. Results indicated that restriction patterns could be used to distinguish among these rodent *Helicobacter* species (Fig. 4).

To determine if a similar approach could be used to distinguish "*F. rappini*" from the rodent *Helicobacter* species, restriction enzyme sites in the amplified region were compared. Results indicated that restriction enzyme analysis with *Mbo*I would differentiate "*F. rappini*" from *H. muridarum* and cleavage with *Hha*I or *Mae*I would distinguish "*F. rappini*" from *H. hepaticus*. However, we did not find a commercially available restriction endonuclease that would distinguish "*F. rappini*" from *H. bilis* (Fig. 3). Therefore, a reverse primer (Hbr) was designed on the basis of the determined *H. bilis* sequence. Five bases at the 3' terminus of this primer were not complementary

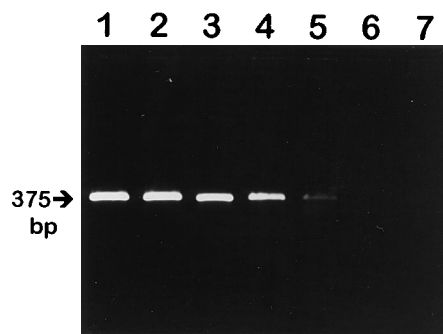


FIG. 2. PCR amplification products from various amounts of *Helicobacter* DNA. Shown is ethidium bromide-stained gel with PCR products resulting from amplification of serial dilutions of *H. hepaticus* in the presence of cecal DNA from uninfected mice. Lanes: 1, 50 ng of DNA; 2, 5 ng of DNA; 3, 500 pg of DNA; 4, 50 pg of DNA; 5, 5 pg of DNA; 6, 500 fg of DNA; 7, no-template control. Analogous data were obtained with DNA from *H. bilis* and *H. muridarum*.

to the corresponding region of the "*F. rappini*" sequence or with the sequences of other known rodent helicobacters (Table 3). PCR amplification with the Hbr and H276f primers amplified *H. bilis* DNA but did not amplify DNA isolated from "*F. rappini*", *H. hepaticus*, or *H. muridarum* (data not shown). The sensitivity of the PCR assay with the H276f and Hbr primers was compared with that of the assay with the genus-specific primers, H276f and H676r, by amplifying 10-fold serial dilutions of *H. bilis*-infected mouse tissue. Comparable results were obtained with the two primer sets, indicating that the sensitivity of the PCR with the *H. bilis*-specific primer set (H276f and Hbr) was similar to that observed with the genus-specific primer set.

**PCR of DNA from tissue samples.** Ten A/JNCr and 10 BALB/c mice were obtained from colonies documented to be infected with *H. hepaticus* and *H. bilis*, respectively. Ten uninfected BALB/c adult male mice served as controls. DNA was isolated from liver and intestinal tissue from each mouse and subjected to PCR evaluation. The expected 374-bp amplification product was detected in 7 of 10 liver DNA samples and 10 of 10 intestinal DNA samples from the *H. hepaticus*-infected A/JNCr mice (Fig. 5). Similar results were obtained with DNA from *H. bilis*-infected mice, with 6 of 10 liver samples and 10 of

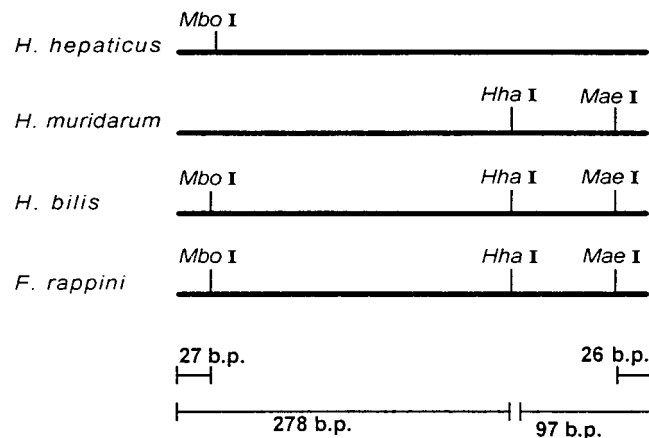


FIG. 3. Restriction map of DNA fragment amplified from rodent *Helicobacter* species with *Helicobacter* genus-specific primers. Sizes of the restriction fragments generated are given in the lower portion of the figure.



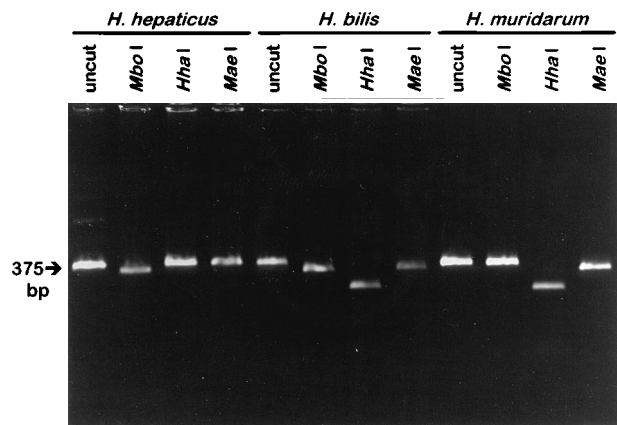


FIG. 4. Restriction enzyme analysis of amplification products. PCR products generated with genus-specific primers and DNA from *H. hepaticus*, *H. muridarum*, and *H. bilis* were digested with *Mbo*I, *Mae*I, or *Hha*I; subjected to agarose gel electrophoresis; and stained with ethidium bromide. Undigested PCR products were included as controls.

10 intestinal samples yielding a PCR product of the expected size (data not shown). Amplified products from *H. hepaticus*- and *H. bilis*-infected mice were sequenced to confirm the specificity of the assay. Evaluation of the results indicated that amplified products were >99.5% homologous to the sequences of *H. hepaticus* and *H. bilis*, respectively.

Using the developed PCR assay system, we examined 508 mouse and 113 rat cecal samples of unknown infection status that were submitted to the University of Missouri Research Animal Diagnostic Laboratory during a 10-month period in 1995 from multiple sources. PCR assays with the genus-specific primers (H276f and H676r) were used to screen all samples. Samples that produced the expected 374-bp fragment were subjected to restriction fragment analysis. Samples that yielded restriction enzyme patterns consistent with either *H. bilis* or "*F. rappini*" were subjected to a second PCR assay with the Hbr and H276f primers to determine if the organism was *H. bilis*. Of the 508 mouse cecal samples examined, 53 (10.4%) were positive for *H. hepaticus*, 87 (17.1%) were positive for *H. bilis*, and 3 (<1%) were positive for *H. muridarum*. No samples were positive for "*F. rappini*." This assay was also used to examine rat cecal samples for the presence of helicobacters. Of the 113 rat tissue samples examined to date, 9 (8.0%) were positive for *H. hepaticus* and 13 (9.7%) were positive for *H. bilis*. No rat cecal samples were positive for either *H. muridarum* or "*F. rappini*."

DISCUSSION

In this report, we describe a DNA amplification assay to detect multiple species of helicobacter organisms in infected mice and rats. Specificity studies indicated that this reaction was specific in that a PCR product of the expected size was generated with DNA from all species of *Helicobacter* organisms tested but was not generated with DNA from other bacterial genera commonly found in mouse intestinal flora. Amplification of DNA from "*F. rappini*" with the *Helicobacter* genus-specific primer set was initially surprising. However, the *Helicobacter* and *Flexispira* genera show a high degree of genetic homology, which has prompted some investigators to propose that "*F. rappini*" should be taxonomically reclassified into the *Helicobacter* genus (13, 14). Inspection of the "*F. rappini*" 16S rRNA gene sequence revealed a 100% match with

our *Helicobacter* genus-specific oligonucleotide primers. Thus, in retrospect, amplification of "*F. rappini*" DNA with these primers should have been expected and reflects the close genetic relationship of "*F. rappini*" with members of the *Helicobacter* genus.

Results described in this study were obtained with a 50-μl reaction volume in a Perkin-Elmer 9600 thermocycler and PCR conditions routinely used in our laboratory, which include short (2-s) denaturation and annealing steps and a 30-s extension step. The fast ramp times of the Perkin-Elmer 9600 thermocycler coupled with the small reaction volume (50 μl) make the short incubation times feasible. Alternatively, reactions can be performed in a Perkin-Elmer 480 thermal cyler in a 100-μl reaction volume with increased incubation times. When the two amplification conditions were compared, no differences in either specificity or sensitivity were noted. The shorter amplification times were selected for routine testing, since they provide a quicker turnaround time for performance of diagnostic PCR.

Restriction enzyme analyses yielded unique patterns for *H. hepaticus* and *H. muridarum*. However, an additional amplification assay with a reverse primer based on *H. bilis* sequence was required to distinguish *H. bilis* from "*F. rappini*." Thus, the combination of PCRs and restriction enzyme analyses provided a method for detecting murine helicobacter infections and identifying the organisms to species level.

In contrast to the PCR assay developed in this study, which detects and identifies multiple *Helicobacter* species, an *H. hepaticus* species-specific PCR assay was recently described by Battles and colleagues (1). The genus-specific assay described in the present report may offer advantages over species-specific PCR assays in diagnostic testing of rodents in that a single PCR can be performed to determine whether the animal is free of helicobacter infection. If amplification of the cecal DNA indicates that the animal is uninfected, no additional testing is required. If the animal is infected, restriction enzyme analysis of the previously generated PCR product and at most one additional PCR assay are needed to detect and identify to species level the bacterial species involved. On the basis of the testing performed in our laboratory, a majority of the mice and rats tested are negative. Thus, diagnostic evaluation of most samples requires only a single PCR assay. In contrast, if one uses species-specific PCR assays, each sample must be evaluated with multiple PCR assays. Thus, use of a genus-specific PCR assay for routine diagnostic testing may minimize testing costs by limiting personnel time and the use of expensive PCR reagents.

Although much of the concern with rodent helicobacters focuses on the hepatitis and hepatic tumors found in infected animals, these organisms are believed to initially colonize the lower intestinal tract. In other ongoing studies in our laboratory, *Helicobacter* organisms were found in naturally infected mice as young as 3 weeks of age, but liver colonization was not detected until mice were 3 months of age. The mechanism for

TABLE 3. Comparison of Hbr primer sequence with DNA sequences of *H. bilis*, "*F. rappini*," *H. hepaticus*, and *H. muridarum*

Primer or organism	Sequence (5' to 3')
Hbr.....	TCTCCCATACTCTAGAAAAGT
<i>H. bilis</i> .....	----- <sup>a</sup>
" <i>F. rappini</i> " .....	-----TGGTCT
<i>H. hepaticus</i> .....	-----C---C---TAAGAG
<i>H. muridarum</i> .....	-----C---A---CTAAT

<sup>a</sup> Dashes indicate sequence homology with the Hbr primer.

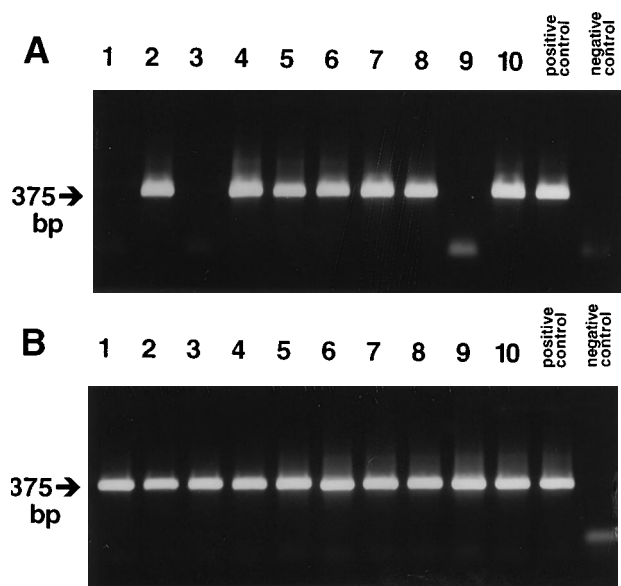


FIG. 5. PCR products generated from amplification of liver (A) and intestinal (B) DNA isolated from 10 mice obtained from a mouse colony endemically infected with *H. hepaticus*. Each lane represents the products from an individual mouse.

translocation of the bacteria from the intestine to the liver is not known. To ascertain whether the liver or the intestine would serve as the optimal tissue for PCR evaluation, we compared the results of PCR assays performed on hepatic and intestinal tissues removed from *H. hepaticus*- and *H. bilis*-infected aged mice in which the liver had already become colonized. Evaluation of the results showed that the intestinal tissue yielded a higher percentage of positive results from naturally infected mice and suggested that the most appropriate target tissue for these assays is the intestine.

Examination of rat and mouse cecal samples submitted to the University of Missouri Research Animal Diagnostic Laboratory for helicobacter screening demonstrated applicability of this approach for identifying rodents infected with *Helicobacter* species. While the samples evaluated do not allow one to draw any definitive conclusions about the prevalence of these organisms in laboratory mice and rats, the results suggest that *H. hepaticus* and *H. bilis* infections are widespread in laboratory mouse and rat colonies. Recognizing the potential detrimental effects *Helicobacter* infections may have on research studies, many investigators are electing to test research animals for *Helicobacter* infections prior to initiating experimental studies to avoid using infected animals. This is particularly true for long-term studies in which the animals must be held for extended periods of time, since the potential for development of hepatic tumors and hepatitis is increased in older animals.

In summary, the developed assay is a sensitive and specific method for detection of helicobacters in cecal tissues. The

assay detects multiple members of the *Helicobacter* genus and appears to be useful in routine diagnostic testing of mice and rats. Evaluation of a large number of laboratory mice and rats demonstrated that *Helicobacter* infections are widespread.

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