Presence of Multiple "Helicobacter heilmannii" Strains in an Individual Suffering from Ulcers and in His Two Cats

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Circumstantial evidence suggests that "Helicobacter heilmannii" infection is an example of zoonosis. The presence of "H. heilmannii" strains in a human subject with acute gastric erosions, in his two cats, and in two unrelated cats was analyzed, and the genetic relatedness of the human and feline strains was assessed. A 580-bp PCR-amplified sequence of "H. heilmannii" urease B gene (ureB) obtained from biopsies from the human subject and his two cats was restricted with AluI and cloned for sequencing. Analysis of the restriction fragment length polymorphism of the ureB-amplified product suggested the presence of different individual "H. heilmannii" strains in the cats and of three distinct strains in the human subject. One of the "H. heilmannii" ureB sequences amplified from the human subject’s biopsies was identical to that derived from one of his cats. The degree of similarity between the other "H. heilmannii" human and feline nucleotide sequences was higher than 97%. Most of the base substitutions were conservative. We conclude that human and animal "H. heilmannii" strains are closely related and that humans can be infected by more than one "H. heilmannii" strain, as has been observed for Helicobacter pylori.

Helicobacter pylori, a stomach-colonizing bacterium that causes gastritis and peptic ulcer disease, is a risk factor for gastric adenocarcinoma (26) and malignant mucosa-associated lymphoid tissue lymphoma (35). Virtually every infected person harbors a different lymphoid tissue lymphoma (35). Virtually every infected person has close contact with animals (31). Studies performed by Stolte et al. demonstrated that "H. heilmannii" infection is an example of zoonosis. The degree of similarity between the other "H. heilmannii" human and feline nucleotide sequences was higher than 97%. Most of the base substitutions were conservative. We conclude that human and animal "H. heilmannii" strains are closely related and that humans can be infected by more than one "H. heilmannii" strain, as has been observed for Helicobacter pylori.

Materials and Methods

Subjects and endoscopy. A 38-year-old dentist presented with a 4-year history of recurrent dyspepsia. Endoscopy showed multiple antral ulcers (10 superficial ulcers up to 6 mm in diameter and 2 mm deep). A rapid urease test on antral biopsies indicated the presence of urease-positive organisms, and histology revealed long, spiral GLO. Endoscopy was performed a second time, and gastric biopsies were done on the antrum and the corpus. The human subject’s two cats (cats 1 and 2) and two cats belonging to different owners (cats 3 and 4) were anesthetized and examined endoscopically as previously described (24).

DNA extraction. DNA extraction was performed by adding 200 µl (to a frozen biopsy) or 400 µl (to a paraffin-embedded biopsy) of K buffer (10 mM Tris-HCl [pH 7.4]–100 mM NaCl–25 mM EDTA–0.5% sodium dodecyl sulfate) containing 100 or 200 mg of protease K per ml. After the tissue was completely dissolved, three phenol-chloroform extractions were performed and DNA was precipitated, dried, and resuspended into 100 µl of sterile distilled water.

Amplification of a ureB 580-bp DNA fragment. The forward primer (5′-GGG CGATAAAAGTGCGTTG-3′ [19-mer]) and the reverse primer (5′-ACCGAGGCGG-3′ [18-mer]) were derived from the published "H. heilmannii" urease B gene. The amplification reaction consisted of 1- to 2-µl DNA samples in a final volume of 50 µl containing 1× PCR buffer (Pharmacia Biotech, Düesseldorf, Switzerland), 200 µM each dNTP, 0.2 µM forward primer, and 2.5 U of Taq DNA polymerase (Pharmacia Biotech, Épalinges, Switzerland). PCR products were cloned into the pGEM-T vector (Promega, Madison, Wisconsin), and 2.5 U of T4 DNA polymerase (Promega, Madison, Wisconsin) was added to the reaction mixture before the addition of the Taq DNA polymerase. Negative results were obtained with the use of this negative control.

FASTA analysis. The PCR products (5 to 15 µl) showing a single band of the expected size were subjected to restriction analysis with the AluI enzyme in the supplied buffer according to the manufacturer’s (Pharmacia) protocol. Restriction products were separated on a 3% agarose gel Containing 0.5 µM ethidium bromide per ml. RFLP analysis. The PCR products (5 to 15 µl) showing a single band of the expected size were subjected to restriction analysis with the AluI enzyme in the supplied buffer according to the manufacturer’s (Pharmacia) protocol. Restriction products were separated on a 3% agarose gel containing 0.5 µM ethidium bromide per ml.
**RESULTS**

PCR amplification and *Alu*I restriction fragment length patterns of a 580-bp DNA fragment of *Helicobacter ureB* in human and cat biopsies. To confirm the morphological evidence suggesting that a 38-year-old human subject was infected by "*H. helimannii*", amplification reactions were performed on DNA extracted from the subject’s gastric antral biopsies with primers shown to be specific for a fragment of the "*H. helimannii*" urease B gene (24). The expected 580-bp amplification product was obtained, unambiguously demonstrating the presence of "*H. helimannii*" in the human subject’s gastric mucosa (Fig. 1A, lane 4); no PCR product could be detected when PCRs were run with primers specific for either *H. pylori* or *Helicobacter felis* ureB (data not shown).

Since "*H. helimannii*" infection in humans is postulated to be transmitted by pets, we performed endoscopy on the human subject’s two cats and established by breath test, rapid urease test, and histology on gastric biopsies that the cats were both infected with GLO, i.e., with urease-positive organisms that resemble "*H. helimannii*" (data not shown). PCRs on DNA extracted from the cats’ gastric biopsies with "*H. helimannii*" ureB-specific primers resulted in a 580-bp product (Fig. 1A, lanes 2 and 3). No amplification was obtained with *H. pylori* or *H. felis*-ureB-derived primers (data not shown).

The human and feline "*H. helimannii*" PCR fragments were then subjected to RFLP analysis with the enzyme *Alu*I (Fig. 1B). Two bands of approximately 310 and 270 bp were obtained from the "*H. helimannii*" PCR fragment from cat 1 (lane 2), three bands of 270, 165, and 145 bp were obtained from the "*H. helimannii*" PCR fragment from cat 2 (lane 3), and a complex pattern was obtained for the human subject’s "*H. helimannii*" fragment (lane 4). The total size of the human subject’s restriction fragments was three times the size of the undigested PCR product, suggesting that he was infected by at least three strains.

**Molecular analysis of the PCR fragments.** To determine the genetic relatedness of the human and cat strains, the 580-bp "*H. helimannii*" ureB PCR products were cloned into the pGEMT vector. Several recombinant clones were obtained, and the nucleotide sequences were determined (Fig. 2). Three different sequences were obtained for the strains from the subject. These sequences were aligned and compared with those for the strains from the human subject’s cats (cats 1 and 2) and with those for the strains of two unrelated cats belonging to different, unrelated owners (cats 3 and 4).

The *Alu*I restriction fragments deduced from the nucleotide sequence data of "*H. helimannii*" strains present in the human subject’s two cats corresponded perfectly to those resolved by RFLP. In contrast, analysis of the sequences of the human subject’s "*H. helimannii*" strains could not explain all the bands visualized by RFLP, suggesting that the 580-bp product amplified from the man’s gastric biopsies contained more sequences than those that were cloned and sequenced.

**Genetic relatedness of human and cat "*H. helimannii*" sequences.** Comparison of the "*H. helimannii*" ureB sequences derived from cats 1 to 4 showed that the sequences were not identical but were nevertheless highly homologous (97.6 to 99.1%). Interestingly, while identity between feline strains was not observed, one of the three sequences derived from the human subject’s biopsy was found to be 100% identical to the sequence from one of his cats (cat 1) (Table 1). Furthermore, the "*H. helimannii*" ureB sequence derived from cat 2 perfectly matched the human sequence found in data banks (EMBL L25079).

When all the sequences were compared, point mutations were observed at 22 different positions along the 580-bp sequence; 16 (73%) occurred in the third base position of the codon, 4 (18%) occurred at the second base position, and 2 (9%) occurred at the first base position. Most of these base substitutions were found to be conservative; i.e., they either did not change the encoded amino acid or substituted a homologous amino acid; indeed, of a total of 6 amino acid substitutions, only two, one in a strain from the human subject (sequence 1b) (E→K188) and one in a strain from cat 4 (H→Y34), were not conservative (Table 2).

These data demonstrate that, despite a significant degree of heterogeneity in the "*H. helimannii*" ureB DNA sequence, amino acid sequences are well conserved and some feline strains are indistinguishable from human strains.

**DISCUSSION**

Colonization by GLO of the gastric mucosa in various pets, especially cats and dogs, has often been described. Thus, in contrast to "*H. pylori*", "*H. helimannii*" has a natural nonhuman reservoir, and transmission from animal to human hosts could occur. The transmission of this bacterium does not seem to be very effective, however, since the prevalence of "*H. helimannii*" is very high in pets (15) but, fortunately, very low in humans (16). In cats and dogs, "*H. helimannii*" colonization is associated with mild to moderate gastritis (6, 24); in humans, it causes mild gastritis (16), but people infected by "*H. helimann-
nii" have also developed gastric ulcers (16, 27, 30, 37) and even cancer (23, 36).

Similarity between the spirilla found in humans and animal bacteria was illustrated by Lee et al. (21). However, despite morphological similarities, it is not clear whether the human "H. heilmannii" strains are identical to those observed in animals. While "H. heilmannii" strains can be maintained in vivo by feeding rodents homogenized gastric biopsies from colonized patients or animals (5, 21), attempts to culture the human and cat "H. heilmannii" in vitro have so far been largely unsuccessful (4). GLO resembling "H. heilmannii" have been described in dogs, but these organisms, named Helicobacter

FIG. 2. Nucleotide sequences of the 580-bp ureB PCR product obtained from human and feline strains. Since the sequence of the "H. heilmannii" ureB fragment from cat 2 was found to be identical to the EMBL 25079 sequence, the nucleotide position refers to the EMBL sequence. Dots denote nucleotide identity. \textit{Alu}I cutting sites used in the RFLP analysis are underlined.
bizzozeronii and Helicobacter salomonis, are culturable, in contrast to “H. heilmannii” (12, 13, 18).

We used PCR to amplify, clone, and sequence a fragment of the “H. heilmannii” urease B gene directly from human and cat gastric biopsies. The structural gene ureB from “H. heilmannii” is highly homologous to ureB from H. pylori and H. felis (29), but we have shown previously that the primers used in our PCRs do not cross-hybridize with DNA from related species and are therefore highly specific (24). The DNA and predicted amino acid sequences reported here are subject to the fidelity constraints of Taq polymerase. The reported measured error rate of Taq polymerase ranges from \(2 \times 10^{-4}\) to less than \(1 \times 10^{-5}\) errors per nucleotide per cycle (7). For the 580 bp of the ureB fragment, we expect \(1\) to \(1\) error per molecule. Therefore, the 7 to 11 base mutations we observed in the 580-bp fragment can truly be attributed to strain heterogeneity and not to Taq polymerase fidelity.

The heterogeneity observed in the “H. heilmannii” ureB sequences (with a DNA sequence similarity ranging between 97.2 and 100%) appears to be comparable to that reported for a partial DNA sequence of ureC from 15 strains of H. pylori (sequence similarity, 95.3 to 99.2%) (9). Together, these data confirm that Helicobacter ureases are encoded by well-conserved genes; other genes such as H. pylori 16S rRNA genes which are already known. We do not know, however, whether this method would have allowed the maintenance of multiple strains, as passage through a different host might lead to the preferential selection of one of the clones.

Although the cats’ owner probably acquired the “H. heilmannii” strain(s) directly from his pet(s), the possibility cannot be excluded that he infected them or that the cats and their owner became infected from the same sources, as other studies have shown that “H. heilmannii” can be found in dogs and pigs (6, 11, 27).

We tried to culture the “H. helimannii” organisms on artificial media but failed. We also tried to maintain and propagate the organisms in vivo by inoculating the human and cat gastric tissues into the stomachs of pathogen-free mice (5). We succeeded with the feline strains but not with the human strains. Propagation of “H. heilmannii” would have allowed us to extend our study and compare the 16S rRNA gene sequences which are already known. We do not know, however, whether this multiple infection was previously known to be possible in humans only in the case of H. pylori (17, 28). Three different sequences were obtained from the human subject in this study, but the restriction profiles obtained were nevertheless unaccounted for. Thus, our data demonstrate (i) that some human and feline “H. heilmannii” strains are very similar, if not identical, suggesting the possibility of transmission between household pets and their owners and (ii) that humans can be infected by more than one “H. heilmannii” strain, as has been observed for H. pylori.

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REFERENCES


TABLE 1. Sequence similarity between the “H. heilmannii” ureB
580-bp fragments from feline and human strains

<table>
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<tr>
<th>Cat no.</th>
<th>% DNA homology for human subject sequence*</th>
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<tr>
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<tr>
<td>2</td>
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<tr>
<td>3</td>
<td>97.6</td>
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<tr>
<td>4</td>
<td>99.1</td>
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* The three sequences (1a, 1b, and 1c) from the human subject were aligned with the four feline sequences (from cats 1 to 4) with the BESTFIT program (Genetics Computer Group).

TABLE 2. Analysis of the nucleotide and inferred amino acid substitutions in the “H. heilmannii” ureB 580-bp fragment

<table>
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<tr>
<th>Source of “H. heilmannii” ureB sequence</th>
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</tr>
<tr>
<td>1a</td>
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* The positions of the amino acids refer to the urease B amino acid sequence derived from sequence EMBL 25079 (ATG→M1).


