Clinical Significance of *Helicobacter* Species Other than *Helicobacter pylori*

Jay V. Solnick
Departments of Internal Medicine (Infectious Diseases) and Medical Microbiology and Immunology, School of Medicine, University of California, Davis

The cultivation of *Helicobacter pylori* and the recognition of its clinical significance have served to stimulate interest in bacteria associated with the gastrointestinal and hepatobiliary tracts. Many novel *Helicobacter* species have been identified and are increasingly recognized in association with human disease, most of which is likely acquired as a zoonosis. Because their identification can be difficult by use of routine methods available in the clinical laboratory, awareness of methods for diagnosis and treatment of these *Helicobacter* species is important, particularly in the evaluation of immunocompromised patients.

Shortly after the identification of *Helicobacter pylori* in 1984, many other helicobacters were described, which now number 22 validated species and a growing list of unvalidated and candidate species [1]. It has become clear over the last 20 years that the stomachs of humans and most other animals have a microbial ecology that consists primarily of one or more urease-producing *Helicobacter* species. Furthermore, other helicobacters are found commonly in the mucus layer of the gut and in the hepatobiliary system of diverse animal hosts. Many, if not all, of these organisms are enzootic in one or more animal hosts, where they commonly cause a local inflammatory response and sometimes are associated with disease. There is now accumulating clinical evidence that many of these *Helicobacter* species also can cause disease in humans, which in most cases probably occurs as a zoonosis. The purpose of this review is to describe the clinical significance, methods of identification, and treatment of these lesser-known *Helicobacter* species, whose contribution to human disease is probably not fully recognized.

**CLINICAL PRESENTATION**

**Gastric disease.** In 1987, a report described a spiral bacterium in human gastric mucosa that was easily distinguished from *Helicobacter pylori* by virtue of its larger size, more tightly coiled morphology, and failure to grow in microaerobic culture (figure 1). Although it was recognized that the organism closely resembled bacteria seen in the stomachs of other animals, it was, nevertheless, initially designated “Gastrospirillum hominis,” which reflected its occurrence in humans. In vitro cultivation of this bacterium is not possible with current methods, so its proper species designation remains controversial. However, 16S rRNA sequence analysis confirmed that it is a *Helicobacter* species, which has been provisionally designated “*H. heilmannii*” [2]. Because it cannot be cultivated, most clinical descriptions of “*H. heilmannii*” rely on morphology for its identification. This practice is convenient for discussion’s sake but probably obscures species differences, because different bacterial species may be morphologically indistinguishable. Indeed, a recent report used in situ hybridization with a 16S rDNA probe to identify 5 “*H. heilmannii*”–like organisms, which are probably distinct, in patient samples [3].

The prevalence of “*H. heilmannii*” among patients who present for endoscopy is <0.5% in most series, including that published shortly before his death by Konrad Heilmann [4], after whom the organism was named. Similar organisms are commonly seen in cats, dogs, pigs, nonhuman primates, and many other animals, which may account for the reports of a prevalence as high as 6% in developing countries, where human
contact with animals may occur more frequently. Gastric mucosal inflammation in patients infected with “H. heilmannii” is less severe than in humans infected with H. pylori [5], but gastritis is, nevertheless, always present. Similar to H. pylori infection, infection with “H. heilmannii” probably is most often asymptomatic. However, infection also has been documented in association with acute and chronic gastrointestinal symptoms, which may resolve with effective therapy, as well as in association with peptic ulcer disease and gastric adenocarcinoma. Recently, remission of gastric mucosa-associated lymphoid tissue (MALT) lymphoma was described in 5 patients after treatment of “H. heilmannii” [6], a phenomenon that is well known in H. pylori–associated MALT lymphoma. Animal models also confirm that “H. heilmannii” causes histological gastritis and MALT lymphoma. A single case report has also described a laboratory worker with an apparent case of gastroenteritis due to H. felis, which is sometimes found in the stomachs of cats and dogs [7].

**Diarrheal disease.** Microbial pathogens that cause diarrhea frequently go unrecognized in clinical evaluations that included routine examination of a stool specimen. Large surveys that use either PCR [8] or sophisticated culture methods [9] may reveal novel bacterial pathogens not previously recognized or underrecognized, in addition to the usual species of Campylobacter, Salmonella, and Shigella. In 1984, a heterogeneous group of Campylobacter-like organisms was first isolated by cultivation of rectal swabs from homosexual men, most of whom had symptoms of proctitis or proctocolitis [10]. Men infected with Campylobacter-like organisms had symptoms that were similar to those infected with C. jejuni, including diarrhea, abdominal cramps, tenesmus, hematochezia, and fever. Most patients had leukocytes, as determined by Gram stain of rectal specimens, and sigmoidoscopic analysis revealed friable mucosa with ulcerations. These observations suggested that novel Campylobacter-like organisms can cause proctocolitis, which may be more common in homosexual men because of anilingus and receptive anal intercourse.

The Campylobacter-like organisms were initially classified into 3 groups on the basis of phenotypic and genotypic characteristics [11]. Subsequent studies showed that 2 of these groups could be classified as novel species, which were designated C. cinaedi (“cinaedi” is Latin for “homosexuals”) and C. fennelliae (after Cynthia Fennell, the technologist who first isolated the organisms), later changed to H. cinaedi and H. fennelliae, respectively [12, 13]. A third group contained only a single isolate, which has not been named and continues to be referred to as “CLO-3.” H. cinaedi is most commonly isolated from homosexual men infected with HIV but also can occur in other immunocompromised patients or, occasionally, in healthy hosts [14]. Enteric infection usually is associated with symptoms that are indistinguishable from those associated with C. jejuni or other causes of inflammatory diarrhea. However, like C. jejuni, H. cinaedi infection also may be asymptomatic. Asymptomatic colonization with H. cinaedi has been found in a wide range of animals; it is best documented in pet hamsters and rhesus monkeys. An animal reservoir of H. fennelliae thus far has not been found but probably exists.

In addition to H. cinaedi and H. fennelliae, which are the helicobacters most commonly associated with enteritis, 5 other helicobacters have been identified in human intestinal infection. H. pullorum was recovered from immunocompetent patients with gastroenteritis, as well as from 1 patient infected with HIV [15]. The organism also was found in the cecal contents of asymptomatic chickens and in the liver and intestines of laying hens with vibriotic hepatitis, which suggests that poultry serves as the source for human infection. Recently, 4 isolates originally classified as H. pullorum, which were obtained by culture from Canadian patients with diarrhea, were shown to belong to a novel species, designated H. canadensis [16]. Whether H. can-
adensis also has an avian reservoir, and is acquired by humans as a zoonosis, has not been determined. Gastroenteritis due to Helicobacter canis [17], which probably was acquired from dogs, and to H. winghamensis [18], whose reservoir is unknown, have also been described. Finally, a heterogeneous group of organisms originally referred to as “Flexispira rappini” has been shown to represent at least 10 different Helicobacter species, which can be part of the fecal flora of dogs, rodents, and other animals. Helicobacters in the “H. rappini” group have sometimes been isolated from patients with diarrhea [19], although their precise species identification remains to be determined.

All these enteric Helicobacter species are closely related to the campylobacters and typically require methods not in routine clinical use to distinguish them. As a result, the prevalence of these infections in humans and in their animal reservoirs remains poorly understood. A possible role for enteric Helicobacter infection in inflammatory bowel disease has been suggested but remains unexplored. Interestingly, severe combined immunodeficient mice reconstituted with CD4+ T cells and infected with H. hepaticus develop severe inflammatory bowel disease that closely resembles that seen in humans [20].

**Bacteremia and disseminated infection.** With the exception of H. canadensis and H. winghamensis, all enteric Helicobacter species that are known to cause diarrhea in humans also have been isolated from blood. Of these, H. cinaedi is most frequently reported, and the clinical syndrome has been described in 2 case series [21, 22]. H. cinaedi bacteremia occurs primarily in immunocompromised hosts, particularly in men infected with HIV. Less commonly, infection may be seen in patients with alcoholism, diabetes, or malignancy and occasionally in patients with no recognized defect in host defense. Nearly all patients have fever, which usually begins abruptly but has a relatively mild, subacute course. Leukocytosis and thrombocytopenia occur variably. Interestingly, colitis is typically not seen in patients with H. cinaedi bacteremia. However, a chronic multifocal cellulitis, usually of the lower extremities, is common. Prolonged bacteremia and recurrent infection after antibiotic therapy have been reported frequently, although without associated mortality. Occasional cases of septic arthritis and meningitis due to H. cinaedi also have been described. A recent report used broad-range 16S rDNA PCR to identify a Helicobacter species closely related to H. cinaedi as the presumptive cause of osteomyelitis in a child [23].

Numerous case reports also describe bacteremia with other enteric helicobacters that cause diarrhea in humans [24]; some of these helicobacters, in fact, may be novel species [25]. Most patients are uncompromised, and there appears to be a particular predilection of H. rappini for patients with X-linked (Bruton’s) agammaglobulinemia [26]. The clinical syndrome of infection with these organisms appears to be indistinguishable from infection with H. cinaedi.

**Hepatobiliary disease.** In 1992, pathologists at the National Cancer Institute recognized that mice serving as saline controls in a long-term chemical carcinogenesis study had a higher-than-expected incidence of liver tumors. Subsequent studies showed that mice with liver tumors also had chronic active hepatitis that could be transmitted to mice without hepatitis that were obtained from a commercial vendor [27]. This strongly suggested an infectious etiology, which was eventually confirmed when Fox et al. [28] succeeded in isolating H. heilmannii from livers, ceca, and colons of affected mice. In some strains of mice, most males infected with H. heilmannii near the time of birth go on to develop hepatocellular tumors between ages 12 and 18 months.

These provocative findings raised the question of whether human hepatocellular tumors and other chronic hepatobiliary diseases might be related to Helicobacter infection. Fox et al. [29] examined frozen bile and resected gallbladders from Chil-ean women with chronic cholecystitis, who have a 30-fold increased risk of gallbladder cancer, compared with that of low-risk populations. Although culture did not yield Helicobacter species, partial 16S rRNA sequences amplified from 8 of 23 patients were nearly identical to those from H. pullorum, H. rappini, or H. bilis. Sequences of 16S rRNA closely resembling that of H. pylori also have been amplified from diverse patients with hepatobiliary disease [30, 31], although, in other cases, the results have been negative [32, 33]. With 1 notable exception [34], Helicobacter species have never been cultivated from human hepatobiliary samples. On balance, it seems likely that Helicobacter species can, at least, transiently colonize the human liver and biliary system. Whether there is persistent infection and whether infection plays a role in disease remain unclear.

**DIAGNOSIS**

**“H. heilmannii.”** The clinical diagnosis of “H. heilmannii” infection requires gastric biopsy with histopathologic examination (figure 1). The organism can be visualized by an experienced pathologist, using either hematoxylin-eosin or Warthin-Starry stains, although the latter is preferable. Touch cytological testing also can be done simply by rolling a gastric biopsy specimen onto a glass slide and staining it with modified Giemsa or Gram stain. Like all known gastric Helicobacter species, “H. heilmannii” makes a potent urease. This can be detected either by biopsy urease test, such as is commonly used to detect H. pylori (e.g., Pyloritek; Horizons International) or by a urea breath test. Neither of these methods will distinguish “H. heilmannii” from H. pylori. Measurement of serum IgG to H. pylori will not detect infection with “H. heilmannii.”

**Isolation of enterohepatic Helicobacter species.** It is unlikely that methods routinely used in clinical microbiology
laboratories will successfully recover enterohepatic helicobacters from feces. The organisms are fastidious and may be overgrown on the plates by more-rapidly growing bacteria. Many species will grow only on freshly poured moist plates incubated in a microaerobic atmosphere enriched with H₂. Furthermore, antibiotics commonly used in selective media for Campylobacter species, such as cephalothin and cefazolin, will inhibit the growth of many enterohepatic Helicobacter species. When helicobacters are isolated, it is often difficult or impossible by means of routine methods to distinguish them from the campylobacters, which are biochemically relatively inert. Some have suggested that optimal recovery of enterohepatic helicobacters may be achieved by plating a fecal slurry onto a 0.45- or 0.65-μm filter that is briefly placed directly onto agar and then incubated under the appropriate conditions [35]. In most instances, recovery and identification of enterohepatic helicobacters from feces is best done in a research laboratory that specializes in these organisms. Samples may be transported frozen in Brucella broth containing 20% glycerol.

In contrast to cultures of feces, enterohepatic Helicobacter species can be isolated from blood in clinical laboratories by use of commercial systems, although the frequency of recovery may be low [21, 22]. Recovery appears to be more frequent and more rapid with the BactT/Alert system (Organon Teknika) than with the Bactec system (Becton Dickinson). Growth is usually apparent only in the aerobic bottles and may require prolonged incubation of 7–10 days or occasionally longer. Blood cultures that develop elevated growth indices should be examined by acridine orange and Giemsa stain, in addition to Gram’s stain, before being discarded as negative.

### Table 1. Helicobacter species other than H. pylori isolated from humans.

<table>
<thead>
<tr>
<th>Species</th>
<th>Clinical disease</th>
<th>Source</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>“H. heilmannii”</td>
<td>MALT lymphoma, peptic ulcer</td>
<td>Stomach</td>
<td>[2–6]</td>
</tr>
<tr>
<td>H. felis</td>
<td>Gastroenteritis</td>
<td>Stomach</td>
<td>[7]</td>
</tr>
<tr>
<td>H. cinaedi</td>
<td>Colitis, cellulitis</td>
<td>Feces, blood</td>
<td>[10–13, 21, 22]</td>
</tr>
<tr>
<td>H. fennelliae</td>
<td>Colitis</td>
<td>Feces, blood</td>
<td>[10–13]</td>
</tr>
<tr>
<td>H. pullorum</td>
<td>Gastroenteritis</td>
<td>Feces</td>
<td>[15]</td>
</tr>
<tr>
<td>H. canadensis</td>
<td>Gastroenteritis</td>
<td>Feces</td>
<td>[16]</td>
</tr>
<tr>
<td>H. winghamensis</td>
<td>Gastroenteritis</td>
<td>Feces</td>
<td>[18]</td>
</tr>
<tr>
<td>“H. rappini”</td>
<td>Colitis</td>
<td>Feces</td>
<td>[19]</td>
</tr>
<tr>
<td>H. canis</td>
<td>Gastroenteritis</td>
<td>Feces</td>
<td>[17]</td>
</tr>
</tbody>
</table>

**NOTE.** For many of these organisms, the associated clinical disease is not well defined. Distinction between colitis and gastroenteritis is somewhat arbitrary, based on the presence of upper gastrointestinal symptoms. MALT, mucosa-associated lymphoid tissue.

### TREATMENT

“H. heilmannii.” Because the prevalence of “H. heilmannii” infection is low, it is unlikely that an etiologic role in gastrointestinal disease will ever be convincingly demonstrated. Nevertheless, it is probably prudent to use the same strategy for treatment of “H. heilmannii” infection that one would use for infection with H. pylori. This is, of course, somewhat controversial, but at the very least one should treat “H. heilmannii” infection in patients with peptic ulcer disease or MALT lymphoma. Whether patients with nonulcer dyspepsia should be treated is controversial, but the preponderance of evidence for H. pylori suggests that there is only marginal benefit. Treatment regimens should include a histamine, blocker or proton pump inhibitor plus at least 2 antibiotics, such as clarithromycin, metronidazole, or amoxicillin, in doses used to treat H. pylori infection. Because “H. heilmannii” cannot be cultivated, in vitro susceptibility studies cannot be done. In general, it appears to be easier to eradicate “H. heilmannii” than H. pylori.

**Enterohepatic Helicobacter species.** There are no guidelines approved by the National Committee for Clinical Laboratory Standards (NCCLS) for methods or break points to determine antibiotic susceptibility of enterohepatic Helicobacter species. Furthermore, as is the case with H. pylori, in vitro susceptibility results may not always correlate with clinical response. Flores et al. [36] determined MICs of 20 antimicrobial agents for 43 strains of H. cinaedi (CLO-1) and 6 strains of H. fennelliae (CLO-2) by means of agar dilution. This method, which has been adopted by the NCCLS for susceptibility testing of H. pylori, is probably preferable to disk diffusion and to the E-test (AB Biodisk). Antimicrobial agents active against all strains tested included ampicillin, gentamicin, doxycycline, tetracycline, chloramphenicol, nalidixic acid, rifampin, and ceftriaxone. These results generally have been confirmed in subsequent case reports, which also have shown that H. cinaedi is usually susceptible to imipenem but not to erythromycin. The in vitro results with ciprofloxacin, which is commonly used for empirical treatment of C. jejuni infection and other causes of bacterial enteritis, have been mixed. Some strains may require a MIC of 0.5–1.0 μg/mL, but others may require MICs as high as 16 μg/mL. Occasional reports have suggested that resistance may develop while the patient is undergoing therapy. The largest clinical experience is with H. cinaedi infection, although the optimal regimen and duration of therapy are unknown. In view of the in vitro results and reports of clinical failures, erythromycin should be avoided, and ciprofloxacin should be used cautiously. For most patients who are not acutely ill, doxycycline is a reasonable choice while waiting for the results of in vitro susceptibility tests, which should be performed on all isolates. For more-seriously ill patients with bacteremia, a broad-spectrum agent, such as imipenem or ceftriaxone plus...
gentamicin, would be appropriate empirical therapy. antimicrobial therapy should be continued for a minimum of 2 weeks and perhaps longer for patients with bacteremia. in view of several reports of recurrent infection, additional blood cultures after completion of therapy should be considered.

conclusions
a growing number of helicobacter species are increasingly being recognized as important human pathogens (table 1), most of which are probably acquired as zoonoses. most important among these presently are gastric infection with "h. heilmannii" and enteric or bloodstream infection with h. cinaedi. the role of helicobacter infection in chronic hepatobiliary diseases is intriguing but as yet unproven. the true extent of ecological niches occupied by these organisms is not yet known, nor has the full spectrum of disease syndromes associated with them been defined. new developments in molecular diagnostics and improved isolation methods not only will better define the role of helicobacter species in human disease but will also reveal novel species not presently recognized.

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