Helicobacter cetorum sp. nov., a Urease-Positive Helicobacter Species Isolated from Dolphins and Whales


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A novel helicobacter with the proposed name Helicobacter cetorum, sp. nov. (type strain MIT 99-5656; GenBank accession number AF 292378), was cultured from the main stomach of two wild, stranded Atlantic white-sided dolphins (Lagenorhynchus acutus) and from the feces of three captive cetaceans (a Pacific white-sided dolphin [Lagenorhynchus obliquidens]; an Atlantic bottlenose dolphin [Tursiops truncatus]; and a beluga whale [Delphinapterus leucas]). The infected captive cetaceans were either subclinical, or clinical signs included intermittent regurgitation, inappetence, weight loss, and lethargy. Ulcers were observed in the esophagus and forestomach during endoscopic examination in two of the three captive animals. In the third animal, esophageal linear erosions were visualized endoscopically, and histopathological evaluation of the main stomach forestomach during endoscopic examination in two of the three captive animals. In the third animal, esophageal linear erosions were visualized endoscopically, and histopathological evaluation of the main stomach

More than 24 Helicobacter species have been identified and named in a wide variety of animals, as well as in humans (13, 39). Members of the genus Helicobacter are microaerobic, have a fusiform or curved to spiral rod morphology, and are motile by flagella that vary in number and location among different species (13, 20, 21, 26, 39, 40). Helicobacters colonize the gastrointestinal tract of humans and animals and are known to cause gastrointestinal disease in various hosts, whereas others appear to be nonpathogenic (13, 20, 30). In humans, Helicobacter pylori is a significant cause of peptic ulcer disease, gastritis, and gastric tumors (25, 39, 40). In animals, Helicobacter spp. may cause ulcerative or nonulcerative gastritis, typhlocolitis, and hepatitis and can lead to tumors in chronic infections (13, 14, 17). The ability of gastric Helicobacter spp. to colonize the stomach is largely dependent on the production of urease (4). Urease hydrolyzes urea into ammonia, resulting in alkalinization of the gastric mucosa, which promotes bacterial colonization and survival (4).

Gastric ulcers have been reported in cetaceans for several decades (8, 33, 37, 38). Parasitic infections have been associated with some lesions, but in other cases no clearly defined etiologies have been identified. We previously described a novel urease-positive Helicobacter sp. cultured from the main stomachs of stranded Atlantic white-sided dolphins that died on the beach in Cape Cod, Mass., and a beluga whale from Mystic Aquarium, Conn. (23, 24). Since then, additional strains of this species were cultured from the feces of a Pacific white-sided dolphin, and an Atlantic bottlenose dolphin from various aquaria in the United States (23). Based on morphological, biochemical, and growth characteristics, as well as 16S RNA gene analysis, the bacteria are classified as a Helicobacter sp. for which we propose the name Helicobacter cetorum sp. nov.

MATERIALS AND METHODS

Animals. The main stomachs from two wild, stranded Atlantic white-sided dolphins were collected at necropsy for culture and PCR analysis. Both animals died on the beach in March 1999 in Wellfleet Cape Cod, Mass. (24). Rectal swabs and gastric fluid also were obtained for culture and PCR from a beluga whale from the Mystic Aquarium in Connecticut (23). In addition, a Pacific white-sided dolphin from the John G. Shedd Aquarium in Chicago and an Atlantic bottlenose dolphin from Dolphin Quest (Oahu, Hawaii) had feces and gastric fluid analyzed (23). A Massachusetts Institute of Technology (MIT) accession number was assigned to each animal, and these numbers will be used throughout the present study (Table 1).

Microaerobic culture of feces and gastric mucosa. Fecal samples for microaerobic culture were collected from the three collection animals (MIT 00-7128, MIT 01-5903, and MIT 01-6202) by inserting a sterile swab (Becton Dickinson, Sparks, Md.) into the rectum and then placing the swab into a 3-ml vial containing brucella broth with 20% glycerol. Samples were then placed on dry ice for transport and stored at −70°C. The media used for culture were Trypticase soy agar with 5% sheep blood, TVP (trimethoprim, vancomycin, and polymyxin), and CVA (cefoperazone, vancomycin, and amphotericin B) antibiotic impregnated media (Remel Laboratories, Lenexa, Kan.). In addition, selective antibiotic medium (ABM) was prepared as follows: blood agar base (Oxoid; Remel), 5% horse blood (Remel), 30 μg of amphotericin B, 100 μg of vancomycin, 3.3 μg of polymyxin B, 200 μg of bacitracin, and 10.7 μg of nalidixic acid (Sigma Chemical Co., St. Louis, Mo./ml. Approximately 0.5 g of feces was homogenized in 1 ml of brucella broth (Difco Laboratories, Detroit, Mich.) containing 5% fetal calf serum (Summit Technologies, Fort Collins, Colo.) in a glass tissue grinder.

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Table 1. Summary of the five cetaceans, with culture and PCR results for Helicobacter infection presented by animal number and sample site

<table>
<thead>
<tr>
<th>Species</th>
<th>Origin</th>
<th>MIT accession(^a) no.</th>
<th>Sample</th>
<th>GenBank no.</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>AWD</td>
<td>Stranded (Mass.)</td>
<td>99-5656</td>
<td>Main stomach</td>
<td>AF292378</td>
<td>+ +</td>
</tr>
<tr>
<td>AWD</td>
<td>Stranded (Mass.)</td>
<td>99-5657</td>
<td>Main stomach</td>
<td>AF292377</td>
<td>+ +</td>
</tr>
<tr>
<td>BW</td>
<td>Mystic Aquarium</td>
<td>00-7128</td>
<td>Feces, gastric fluid</td>
<td>AF455130</td>
<td>+ +</td>
</tr>
<tr>
<td>PWD</td>
<td>John G. Shedd Aquarium</td>
<td>01-5903</td>
<td>Feces, gastric fluid</td>
<td>AY143175</td>
<td>+ +</td>
</tr>
<tr>
<td>ABD</td>
<td>Dolphin Quest</td>
<td>01-6202</td>
<td>Feces</td>
<td>AY143177</td>
<td>+ +</td>
</tr>
</tbody>
</table>

\(^a\) AWD, Atlantic white-sided dolphin; BW, beluga whale; PWD, Pacific white-sided dolphin; ABD, Atlantic bottlenose dolphin.

A total of 100 μl of each sample was applied directly to TVP, CVA, and ABM media. Half of the remaining portion of the sample was filtered through a 0.45-μm (pore-size) filter onto a blood agar plate. The plates were incubated at 37°C under microaerobic conditions for 2 to 4 weeks in vented jars containing N₂, H₂, and CO₂ (98:10:10). The gastric mucosa from the main stomachs (second chamber) of two stranded Atlantic white-sided dolphins were collected in the field at necropsy (MIT 99-5656 and MIT 99-5657), and cultures were performed as previously described (24).

Biochemical characterization of bacterial isolates. To further characterize the bacterial isolates, biochemical and phenotypic tests commonly employed to characterize helicobacters were performed using a previously described protocol (21, 26).

DNA extraction and PCR analysis from gastric fluids and fecal samples. Fecal samples and gastric fluids for PCR analysis were collected from the three collection animals (MIT 00-7128, MIT 01-5903, and MIT 01-6202). The gastric fluid was collected by endoscopy by using a CF-140L Video colonoscope measuring 268 cm (Olympus America, Melville, N.Y.) and divided into aliquots in individual dram vials. The endoscope and its channels were rinsed sequentially in dilute detergent solutions and fecal samples and gastric fluids were collected as described above, and the samples were placed in empty sterile vials. DNA was extracted from the fecal samples and gastric fluids with a modified Mini QIAamp DNA kit (Qiagen).

DNA extraction and PCR analysis from cultured bacteria. DNA was extracted from cultured bacteria with the High-Pure PCR template preparation kit (Roche Molecular Biochemicals, Indianapolis, Ind.). The Helicobacter sp.-specific primer pair C97 and C05 was used to generate 16S rRNA gene amplicons of 1,200 bp (Table 2) (17, 23). Then, 10 μl of the DNA preparation was used for PCR. The PCR mixture (100 μl) contained 1× Taq polymerase buffer, 0.5 μM concentrations of each of the two primers, 200 μM concentrations of each deoxynucleotide, and 200 μg of bovine serum albumin/ml, and 2.5 μl of Taq polymerase (Roche Molecular Biochemicals) was also added. Amplification conditions were as follows: denaturation at 94°C for 1 min, annealing at 58°C for 2 min, and elongation at 72°C for 3 min. Thirty-five cycles were completed before a final elongation step at 72°C for 8 min. A 15-μl aliquot of the PCR product was examined by electrophoresis in a 1% agarose gel separation matrix. The DNA was stained with ethidium bromide, and viewed under a UV light. PCR products were purified with a QIAquick PCR purification kit (Qiagen).

RFLP analysis. Helicobacter sp.-specific 1,200-bp PCR products for the 16S rRNA gene were subjected to restriction fragment length polymorphism (RFLP) analysis. DNA was digested in separate reactions with the restriction endonucleases AluI and HhaI (New England Biolabs, Beverly, Mass.). Each reaction contained 10 μl of either AluI or HhaI, 2 μl of restriction buffer (New England Biolabs), and 16 μl of PCR product. Reactions were incubated at 37°C for 2 h. The products were examined by electrophoresis through a 6% Visigel separation matrix (Stratagene, La Jolla, Calif.), stained with ethidium bromide, and viewed under UV illumination.

Amplification of 16S rRNA cistrons by PCR and purification of PCR products from cultured bacteria. The 16S rRNA cistrons from the gastric mucosa culture isolates (MIT 99-5656 and MIT 99-5657) and fecal culture isolates (MIT 00-7128, MIT 01-5903, and MIT 01-6202) were amplified with universal bacterial primers F24 and F25 (Table 2) (10). Hot start PCR was performed in thin-walled tubes with a Perkin-Elmer 9700 Thermocycler. Next, 1 μl of the DNA template was added to a reaction mixture (50 μl, final volume) containing 20 pmol of each primer, 40 mmol of deoxynucleotide triphosphates, and 1 U of Taq 2000 polymerase (Stratagene) in buffer containing Taqstart antibody (Sigma). In a hot-start protocol, samples were preheated at 95°C for 8 min, followed by amplification under the following conditions: denaturation at 95°C for 45 s, annealing at 60°C for 45 s, and elongation for 1.5 min with an additional 5 s for each cycle. A total of 30 cycles were performed, followed by a final elongation step at 72°C for 10 min. The PCR amplicons were examined by electrophoresis in a 1% agarose gel. DNA was stained with ethidium bromide and visualized under short-wavelength UV light. PCR products were purified with a QIAquick PCR purification kit (Qiagen).

16S rDNA gene sequencing and data analysis. Purified DNA from PCR was sequenced with an ABI Prism cycle-sequencing kit (BigDye terminator cycle sequencing kit with AmpliTaq DNA polymerase FS [Perkin-Elmer]). The primers used for sequencing were previously described (10, 16). Quarter-dye chemistry was used with 80 μM concentrations of primers and 1.5 μl of PCR product in a final volume of 20 μl. Cycle sequencing was performed with an ABI 3700 DNA sequencer with 25 cycles of denaturation at 96°C for 10 s and annealing and extension at 60°C for 4 min. Sequencing reactions were run on an ABI 377 DNA sequencer. Sequence data were entered into RNA, a program set for data entry, editing, sequence alignment, secondary structure comparison, similarity matrix generation, and dendrogram construction for 16S rDNA in Microsoft QuickBasic for use with PC computers and were aligned as previously described (31). Our database contains >1,000 sequences obtained in our laboratory and >500 re-

<table>
<thead>
<tr>
<th>Primer</th>
<th>Position (nucleotide range)</th>
<th>Orientation</th>
<th>PCR specificity</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>F24</td>
<td>9–27</td>
<td>Forward</td>
<td>Universal</td>
<td>GAGTTGTATGTTTTGCTACAG</td>
</tr>
<tr>
<td>F25</td>
<td>1525–1541</td>
<td>Reverse</td>
<td>Universal</td>
<td>AAGAGGATTGTCACARCC</td>
</tr>
<tr>
<td>C97</td>
<td>276–291</td>
<td>Forward</td>
<td>Helicobacter</td>
<td>GCTATGACGGGTATCC</td>
</tr>
<tr>
<td>C05</td>
<td>1477–1495</td>
<td>Reverse</td>
<td>Helicobacter</td>
<td>ACTTCACCCAGTGCTGT</td>
</tr>
</tbody>
</table>

\(^a\) Primers F24 and F25 were used for PCR of genomic DNA for cycle sequencing. Primers C97 and C05 were used to amplify a 1.2-kb product from fecal samples for cloning.

Table 2. PCR primers used
The GenBank accession numbers for the strains used in the present study are listed in Table 1 (see also Fig. 4). The biopsies were obtained from the first and second gastric chambers of the Pacific white-sided dolphin (MIT 01-5903). The biopsies were immediately frozen for PCR, while one biopsy each from the first and second gastric chambers were immediately placed in 10% neutral buffered formalin. Formalin-fixed tissues were processed for histopathology, sectioned at 4 to 5 μm, and stained with hematoxylin and eosin (H&E). Sections of the second gastric chamber were also stained with a modified Steiner's silver stain (6).

Nucleotide sequence accession numbers. The GenBank accession numbers for the strains used in the present study are listed in Table 1 (see also Fig. 4). The 16S rRNA gene sequence of *H. cetorum* MIT 99-5656 was deposited in GenBank under accession number AF292378.

**RESULTS**

**Animals.** The beluga whale (MIT 00-7128) had clinical signs of intermittent inappetence and lethargy (23). The Atlantic bottlenose dolphin (MIT 01-6202) had chronic regurgitation, with evidence of gastric inflammation based on cytology of gastric fluid. Endoscopic examination of the beluga whale and Atlantic bottlenose dolphin revealed esophageal and forestomach ulcers. The main stomach was not visualized in these two animals due to the size of the animal or to limited accessibility of the second chamber. The Pacific white-sided dolphin (MIT 01-5903), although asymptomatic at the time of sampling, had a recent history of chronic regurgitation and weight loss and, upon endoscopic examination, linear erosions of the esophageal mucosa were visualized.

**Microaerobic culture and biochemical characterization of the novel *Helicobacter* sp.** *Helicobacter* sp. was cultivated from the glandular mucosa in two stranded animals (MIT 99-5656 and MIT 99-5657) and from the fces of the three collection animals: the beluga whale (MIT 00-7128), the Pacific white-sided dolphin (MIT 01-5903), and an Atlantic bottlenose dolphin (MIT 01-6202) (9, 23). Cultures on solid media were visible after incubation for 2 to 4 weeks under microaerobic conditions as a thin, spreading film. Once pure cultures of the fusiform phenotype were isolated, subsequent passages yielded growth on blood agar plates by 2 to 5 days at 37°C. By light microscopy, the morphological phenotypes of the bacteria were all gram negative, motile, and fusiform. The novel *Helicobacter* isolates were negative for nitrate reduction, alkaline phosphatase hydrolysis, and indoxyl acetate hydrolysis. All isolates grew under microaerophilic conditions at 37 and 42°C but not at 25°C. All isolates were susceptible to cephalothin (Table 3). However, isolates collected from the wild stranded Atlantic white-sided dolphins were sensitive or intermediate to nalidixic acid, but the isolates from the three captive animals were all resistant.

**PCR analysis of gastric tissue, gastric fluid, and feces.** DNA from the stomach tissues of two stranded dolphins and DNA from the feces and gastric fluids of three collection animals was amplified with a *Helicobacter* species-specific primer set. Of the eight samples analyzed by PCR, all were positive (Table 1). The main stomachs of the two stranded Atlantic white-sided dolphins (MIT 99-5656 and MIT 99-5657) and the gastric fluids and feces from the three collection animals (MIT 00-7128, MIT 01-5903, and MIT 01-6202) were all positive for the presence of *Helicobacter* sp. (Table 1).

**PCR analysis of the novel *Helicobacter* isolate and RFLP.** The five culture isolates (two from gastric tissue and three from fecal samples) yielded a 1,200-bp PCR product with *Helicobacter*-specific primers (Fig. 2). The matching patterns observed for all five bacterial 16S rRNA gene PCR products subjected to RFLP analysis with *HhaI* and *AluI* digestion indicates that the five cetacean helicobacter types are the same. Fragment sizes were as predicted by 16S rRNA sequence data (Fig. 3).
Sequencing and phylogenetic analysis of a novel *Helicobacter* sp.

Full 16S rRNA gene sequences were obtained from the five bacterial isolates cultured from four dolphins and one beluga whale (MIT 99-5656, MIT 99-5657, MIT 00-7128, MIT 01-5903, and MIT 01-6202). A dendrogram illustrating the relationship of these strains to selected *Helicobacter* species is shown in Fig. 4. All sequences form a monophyletic cluster. The sequences, except MIT 99-5657, differ among the isolates by only two to five bases and thus appear to represent the same species. Strain MIT 99-5657 differs from the other strains by 18 bases.

TABLE 3. Biochemical characteristics of cetacean *H. cetorum* compared to other gastric *Helicobacter* spp.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Species</th>
<th>Catalase production</th>
<th>Urease activity</th>
<th>Nitrate reduction</th>
<th>Indoxyl acetate hydrolysis</th>
<th>Motility</th>
<th>Anaerobic</th>
<th>Cephalothin (30-mg disk)</th>
<th>Nalidixic acid (30-mg disk)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. cetorum</em></td>
<td>MIT 99-5665</td>
<td>AWD</td>
<td>/H11001/H11001</td>
<td>/H11002/H11002</td>
<td>/H11001/H11002</td>
<td>S</td>
<td>S</td>
<td>/H11001/H11001</td>
<td>/H11001/H11002</td>
</tr>
<tr>
<td><em>H. cetorum</em></td>
<td>MIT 99-5657</td>
<td>AWD</td>
<td>/H11001/H11001</td>
<td>/H11002/H11002</td>
<td>/H11001/H11002</td>
<td>S</td>
<td>S</td>
<td>/H11001/H11001</td>
<td>/H11001/H11002</td>
</tr>
<tr>
<td><em>H. cetorum</em></td>
<td>MIT 00-7128</td>
<td>BW</td>
<td>/H11001/H11001</td>
<td>/H11002/H11002</td>
<td>/H11001/H11002</td>
<td>S</td>
<td>S</td>
<td>/H11001/H11001</td>
<td>/H11001/H11002</td>
</tr>
<tr>
<td><em>H. cetorum</em></td>
<td>MIT 01-5903</td>
<td>PWD</td>
<td>/H11001/H11001</td>
<td>/H11002/H11002</td>
<td>/H11001/H11002</td>
<td>S</td>
<td>S</td>
<td>/H11001/H11001</td>
<td>/H11001/H11002</td>
</tr>
<tr>
<td><em>H. cetorum</em></td>
<td>MIT 01-6202</td>
<td>ABD</td>
<td>/H11001/H11001</td>
<td>/H11002/H11002</td>
<td>/H11001/H11002</td>
<td>S</td>
<td>S</td>
<td>/H11001/H11001</td>
<td>/H11001/H11002</td>
</tr>
<tr>
<td><em>H. pylori</em></td>
<td>Human</td>
<td>/H11001/H11001</td>
<td>/H11002/H11002</td>
<td>/H11001/H11002</td>
<td>/H11001/H11002</td>
<td>S</td>
<td>R</td>
<td>/H11001/H11001</td>
<td>/H11001/H11002</td>
</tr>
<tr>
<td><em>H. acinonychis</em></td>
<td>Cheetah</td>
<td>/H11001/H11001</td>
<td>/H11002/H11002</td>
<td>/H11001/H11002</td>
<td>/H11001/H11002</td>
<td>S</td>
<td>R</td>
<td>/H11001/H11001</td>
<td>/H11001/H11002</td>
</tr>
</tbody>
</table>

a AWD, Atlantic white-sided dolphin; BW, beluga whale; PWD, Pacific white-sided dolphin; ABD, Atlantic bottlenose dolphin.

b MIT, Massachusetts Institute of Technology, Cambridge, Mass.

c S, sensitive; R, resistant; I, intermediate.
FIG. 4. Dendrogram depicting the phylogenetic location of the *H. cetorum* constructed on the basis of the 16S rRNA sequence similarity values. The sequences from the Atlantic white-sided dolphins (MIT 99-5656 and MIT 99-5657), beluga whale (MIT 00-7128), a Pacific white-sided dolphin (MIT 01-6202), and an Atlantic bottlenose dolphin (MIT 01-5903) are identified with arrows. The number in parentheses after the MIT accession number is the GenBank accession. The scale bar is equal to a 3% difference in nucleotide sequences, as determined by measuring the lengths of the horizontal lines connecting two species.

The cetacean sequences cluster with the gastric helicobacters (*H. pylori* and *H. acinonychis*). The five cetacean isolates are most closely related to *H. pylori*, differing by only 1.8 to 2.1%. Electron microscopy. The bacteria isolated from the wild, stranded Atlantic white-sided dolphin (MIT 99-5656) measured 0.6 by 4 μm and are slightly spiral with bipolar sheathed flagella that are laterally located at the end of the bacteria (Fig. 1) (24).

Histopathology. The gastric biopsy from the glandular main stomach (second gastric chamber) of the Pacific white-sided dolphin (MIT 01-5903) revealed two, small, discrete aggregates of lymphocytes with few admixed plasma cells in the lamina propria and mild compression and distortion of the surrounding glands (Fig. 5). Scattered lymphocytes and plasma cells were noted throughout adjacent lamina propria. Multifocal glands both immediately adjacent and distant to the lymphoid aggregates contained rare to numerous, up to 4 μm long, ca. 1 μm wide, loosely coiled spiral bacteria evident on both H&E- and Steiner-stained slides (Fig. 6). These lesions mimic the lesions previously described in the main stomach of stranded dolphins (24). The biopsy of the nonglandular stomach (first gastric chamber) was within normal limits.

**DISCUSSION**

Numerous *Helicobacter* species have been described from humans, as well as terrestrial animals. However, to our knowledge, this is the first *Helicobacter* sp. from a marine mammal to be characterized and named. This organism has been identified in cetaceans belonging to the suborder Odontoceti and the families *Mondodontidae* and *Delphinidae*. By 16S rRNA gene sequence analysis, *H. cetorum* sp. nov. clusters with *H. pylori* and other gastric *Helicobacter* species. Interestingly, the *Helicobacter* strain of *H. cetorum* characterized from the captive Hawaiian dolphin was identical to the wild and captive cetaceans from the east coast of the United States.

The PCR results for the feces and gastric fluid from the three captive cetaceans were positive for *H. cetorum* as were fecal cultures. It has been reported that direct detection of *Helicobacter* spp. in feces can be limited by fecal inhibitors, such as bile, which may lead to false-negative results (25, 27, 28). The ability to culture the bacteria from the feces suggests that fecal-oral transmission may be important in the epizootiology of this infection in cetaceans. In other species, such as *H. pylori*, fecal shedding, as well as waterborne routes of transmission, have been postulated as modes of transmission (1, 12, 30; A. P. West, A. P., M. R. Miller, and D. S. Tompkins, Letter, J. Clin. Pathol. 43:609, 1990). Results from ferrets that have been used as an animal model to study the mode of transmission of gastric helicobacters support the concept of fecal-oral transmission (3). These studies suggest that hypochlorhydria and rapid gastrointestinal transit time in *Helicobacter mustelae*-infected ferrets is an important factor that promotes fecal shedding (15, 18, 19).

All three captive dolphins had different clinical presentations. The beluga whale (MIT 00-7128) manifested intermittent inappetence, and lethargy and endoscopy revealed esophageal and forestomach ulcers. The clinical history of the Atlantic bottlenose dolphin (MIT 01-6202) included chronic regurgitation with evidence of gastric inflammation based on cytology of gastric fluid. Endoscopic examination of the bottlenose dolphin revealed esophageal and forestomach ulcers. The main stomach (second chamber) was not visualized in either of these two animals. The Pacific white-sided dolphin (MIT 01-5903) had a clinical history of chronic regurgitation and weight loss. The endoscopic examination of the Pacific white-sided dolphin revealed linear erosions of the esophageal mucosa, which were attributed to reflux of gastric juices subsequent to chronic regurgitation. Although chronic regurgitation is historically attributed to abnormal behavior, the histopathological findings, including the presence of spiral
bacteria in glandular tissue in these dolphins, are consistent with those associated with gastric *Helicobacter* infection in other species and provide an alternative hypothesis to clinical signs displayed in these animals (13, 14).

Cetaceans have a three-chambered stomach composed of the nonglandular forestomach and the glandular main and pyloric stomachs (22, 34, 37). In wild and captive cetaceans, ulcers have been reported in both the nonglandular and the glandular stomachs (37, 38). Technical limitations usually prevent routine endoscopic examination of the second chamber (i.e., the main stomach) in cetaceans. Our previous studies indicated that helicobacters colonize the glandular main and pyloric stomach in dolphins but do not colonize the nonglandular forestomach (24).

Similar to cetaceans, pigs have a well-demarcated glandular and nonglandular portion in their stomach (7, 11). In swine, gastric ulcers are identified in the nonglandular pars esophagea (7). Although the etiopathogenesis of gastric ulcer disease in pigs remains unclear, investigators have recently suggested that gastric lesions in the nonglandular stomach in pigs may be related to the presence of helicobacters in the glandular stomach (2, 32, 35).

Since the first report of a novel gastric helicobacter in dolphins in 1999, *Helicobacter* spp. have been detected by PCR analysis in more than 30 different wild and captive dolphins around the United States. Wild species include Atlantic bottlenose dolphins, Atlantic white-sided dolphins, and common dolphins. Captive species include Atlantic bottlenose dolphins, Pacific white-sided dolphins, and a beluga whale. In several of these cases, RFLP analysis of the *Helicobacter* sp.-specific PCR products was performed, and the patterns generated were similar to the patterns seen with *H. cetorum* (C. G. Harper and J. G. Fox, unpublished data) (23, 24). Most of the animals with known clinical histories had signs consistent with gastrointestinal disease. Clinical signs varied, but each case included at least one of the following: gastric lesions or ulcers noted on endoscopy, lethargy, inappetance, or regurgitation. To date, we are unable to conclude that *H. cetorum* is the etiological agent responsible for the development of gastric ulcer disease in cetaceans due to the limited access to gastric biopsies for detailed histopathology, and the inability to fulfill Koch’s postulates in cetaceans. However, based on culture and histopathological analysis of inflamed gastric tissue of cetaceans, *H. cetorum* appears to be associated with gastritis. Analysis of additional animals and eradication of *H. cetorum* in infected animals, with subsequent remission of gastric disease and clinical signs, are necessary for establishing whether *H. cetorum* plays a role in gastric ulcer disease in cetaceans.

The proposed species *H. cetorum* differs from named species by several different criteria. First, it is the first named species
isolated from aquatic mammals. Second, unlike other gastric Helicobacter spp., *H. cetorum* is negative for alkaline phosphatase and intermediate in susceptibility to nalidixic acid. Finally, the lateral location of the flagella in *H. cetorum* is seen only with one other helicobacter, *H. mustelae*, whose rod-shaped morphology easily distinguishes it from *H. cetorum*, as well as from other gastric helicobacters (29).

**Description of Helicobacter cetorum, sp. nov.** *Helicobacter cetorum* (ce.to’rum) L. plur. gen. n. cetorum of cetaceans (whales, dolphins). Cells are fusiform with no periplasmic fibers and measure 0.6 by 4 μm. Bipolar single flagella account for the motility of the bacterium. Older cultures contain large coccoid forms. Growth on agar plates appears as a thin, spreading film; distinct colonies are present. Microaerobic growth occurs at 37 and 42°C but not at 25°C. Brucella agar plates containing 1% glycine do not support growth. No growth is seen under aerobic and anaerobic conditions. Cells are positive for urease, catalase, oxidase, and gamma-glutamyl transpeptidase activities. Tests for indoxyl acetate hydrolysis, nitrate reduction, and alkaline phosphatase hydrolysis are negative. The bacteria have intermediate sensitivity to nalidixic acid but are sensitive to cephalothin. Cells were isolated from the stomachs and feces of adult captive cetaceans. The type strain, MIT 99-5656, from an Atlantic white-sided dolphin has

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**FIG. 6.** Photomicrograph of the main stomach (antrum) tissue of a Pacific white-sided dolphin (MIT 01-5903). Numerous ca. 2-μm by 5- to 8-μm curved and spiral argyrophilic bacteria are evident in the surface mucous layer (Steiner staining; magnification, ×250).
been deposited with the American Type Culture Collection as ATCC BAA-540 and the beluga isolate (MT 00-7128) has been deposited as ATCC BAA-429.

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