Fluorescence In Situ Hybridization Confirms Clearance of Visible *Helicobacter* spp. Associated with Gastritis in Dogs and Cats


**Background:** The results of studies examining the role of *Helicobacter* spp. in the pathogenesis of canine and feline gastritis are inconclusive. Furthermore, data evaluating the effectiveness of medical therapy for eradication of *Helicobacter* infection are limited.

**Aim:** To detect *Helicobacter* spp. in mucosal biopsies of dogs and cats diagnosed with gastritis, with fluorescence in situ hybridization (FISH).

**Animals:** Three dogs and 2 cats with signs of chronic gastrointestinal disease.

**Methods:** Dogs and cats infected with *Helicobacter* spp. were treated with triple antimicrobial therapy and fed an elimination diet for 21 days. *Helicobacter* spp. status in endoscopic (3 dogs, 1 cat) or surgical biopsies (1 cat) of gastric mucosa was compared pre- and posttreatment in each animal by histology, FISH analysis, and polymerase chain reaction (PCR).

**Results:** Gastritis of varying severity with intraglandular spiral bacteria was observed in all animals. Pretreatment diagnostic tests confirmed the presence of mucosal *Helicobacter* spp. in all animals by FISH and histopathology and in 4/5 animals by PCR. Rapid resolution of vomiting episodes was observed in all animals. Gastric biopsies performed after triple therapy revealed clearance of visible *Helicobacter* spp. by histopathology and negative FISH analysis, as well as PCR in all animals.

**Conclusions and Clinical Importance:** Application of FISH to routine biopsy specimens enabled rapid and specific identification of *Helicobacter* spp. within the gastric mucosa of dogs and cats. Although medical therapy was useful in resolution of clinical signs and clearance of visible *Helicobacter* spp. in gastric biopsies, gastric inflammation persisted.

**Key words:** 16S rDNA; Gastric disease; Triple therapy.

*Helicobacter* spp. infections in humans are associated with substantial morbidity and mortality owing to peptic ulcers and gastric neoplasia. *Helicobacter pylori*-positive patients have a 10–20% lifetime risk of developing ulcer disease and a 1–2% risk of developing distal gastric cancer. Owing to the associated morbidity and mortality, eradication therapy is recommended in clinically symptomatic humans with a combination of antibiotics and antisecretory drugs. This treatment results in clinically symptomatic humans with a combination of antibiotics and antisecretory drugs. This treatment results in resolution of vomiting episodes in all animals. Gastric biopsies performed after triple therapy revealed clearance of visible *Helicobacter* spp. by histopathology and negative FISH analysis, as well as PCR in all animals.

Additionally, most therapeutic studies in dogs and cats have not revealed long-term eradication of *Helicobacter* spp. without this result is owing to reinfection or recurrence has not been established.

Fluorescence in situ hybridization (FISH) is a relatively new and integral part of culture-independent assessment of microbial ecology. Fluorescence-labeled 16S rDNA-targeted oligonucleotide probes have become a widely used technique for detection of whole bacteria in their natural habitat by fluorescence microscopy of prepared specimens (ie, the gastric mucosa of infected humans or animals). FISH combines the molecular identification of bacteria with direct visualization of the relationship between the bacteria and the mucosa, providing a substantial advantage over culture, PCR, and histologic methods (eg, Steiner and Warthin-Starry staining) alone. Indeed, FISH is more specific than silver staining because it may be tailored to accurately discriminate between bacteria on the basis of 16S or 23S sequences rather than the ability of these bacteria to pick up a silver stain. FISH recently has been used to visualize the intragastric distribution of *Helicobacter* spp. in healthy and diseased dogs and cats. This study investigated the use of FISH for detection of *Helicobacter* spp. in gastric biopsy specimens from 3 dogs and 2 cats with chronic gastritis, and the effects of triple therapy and an elimination diet on clearance of visible organisms and attenuation of mucosal inflammation.

**Methods**

**Clinical Evaluation**

Study animals were referred (2003–2007) to the Iowa State University VTH for clinical evaluation of chronic (> 3-week duration) gastroenteritis. Before study inclusion, each animal had been fed 1 or more commercial elimination (intact protein) diets for at least 30 days and had received treatment with broad-spectrum anthelmintics for gastrointestinal parasites. One dog and 1 cat had...
been given antibiotics (ie, metronidazole for 5–7 days) for gastritis but vomiting frequency did not change dramatically. None of the animals had been treated with antibiotics, corticosteroids, or antacids in the 2 weeks before entering the study. The minimal diagnostic evaluation performed in each animal included a CBC, serum biochemistry profile, urinalysis, direct and indirect examination of feces for parasites, and survey abdominal radiographs. In some instances, abdominal ultrasonography (n = 2 cats), serum pancreatic-like immunoreactivity (n = 2 cats), serum folate and cobalamin concentrations (1 dog, 1 cat), or some combination of these diagnostic tests also was performed as deemed appropriate. Clinical evaluation was repeated in each animal after completion of triple therapy for gastric Helicobacter spp. infection. Posttreatment examinations were not performed at the same time in all animals owing to financial constraints and weather-related delays in traveling to the VTH.

Gastrointestinal Endoscopy and Histopathology

Multiple (8–12 per organ evaluated) mucosal biopsy specimens were obtained endoscopically from the stomach (fundus, gastric body, and antrum) and small intestine of diseased animals at diagnosis and after 21 days of drug therapy. Posttreatment mucosal biopsies were obtained no earlier than 4 weeks (mean, 6.9 weeks; range, 4–14 weeks) after cessation of drug therapy. Biopsy specimens were placed on ethanol-fixed cucumber slices (to ensure proper orientation after tissue sectioning), immersed in neutral-buffered formalin, and processed routinely. Sections were stained with hematoxylin and eosin (H&E) and Warthin-Starry stains and buffered formalin, and processed routinely. Sections were stained with hematoxylin and eosin (H&E) and Warthin-Starry stains and immersed in neutral-buffered formalin, and processed routinely. Sections were stained with hematoxylin and eosin (H&E) and Warthin-Starry stains and examined by 1 of the 2 pathologists (ISH, JDS).

Triple Therapy for Gastric Helicobacter spp. Infection

Standardized drug therapy consisting of oral metronidazole (11–15 mg/kg PO q12h), amoxicillin (22 mg/kg PO q12h), and bismuth subsalicylate suspension (0.22 mL/kg PO q6–8h) administered for 3 weeks was used in each animal. This same therapy had previously revealed efficacy in the eradication of gastric helicobacters in dogs.9 This therapy was repeated in each animal after completion of triple therapy for gastric Helicobacter spp. infection. Posttreatment examinations were not performed at the same time in all animals owing to financial constraints and weather-related delays in traveling to the VTH.

FISH

Formalin-fixed paraffin-embedded histologic sections (4 μm) of the stomach were evaluated by FISH as described previously. In brief, paraffin-embedded biopsy specimens were deparaffinized by passage through xylene (3 × 10 minutes), 100% alcohol (2 × 5 minutes), 95% ethanol (5 minutes), and lastly 70% ethanol (5 minutes). Once the slides were air-dried, FISH probes 5'-labeled with either Cy-3 or 6-FAM were reconstituted with sterile water and diluted to a working concentration of 5 ng/μL with a hybridization buffer appropriate for the probe (Table 1). The specific formamide concentration used in the hybridization buffer for individual probes varied between 35 and 40%. A EUB 338 Cy-3 probe, specific for all bacteria (Table 1), was combined with the nonsense probe non-EUB 338-FAM (ACT CCT ACG GGA GGC AGC) to assess for non-specific hybridization.21 A combination of 2 different carbocyanine (Cy-3)-labeled probes specific for Helicobacteraceae (Table 1) was used to determine if these bacteria were present in the gastric mucosa.22 For subsequent analysis, the Helicobacteraceae-specific probes labeled with Cy-3 and the universal bacterial probe Eubacteria (EUB) 338 labeled with 6-arboxyfluorescein (6-FAM) were applied simultaneously. Tissue sections also were counterstained with 4,6-diamidino-2-phenylindole (DAPI) to visualize DNA. Sections were allowed to hybridize with 30 μL of the DNA probe mixture in a hybridization chamber overnight (46°C for 12–14 hours). Slides next were washed with an appropriate wash buffer (hybridization buffer without sodium dodecyl sulfate), the samples were rinsed in sterile water, allowed to air-dry, and mounted with Prolong Antifade Gold.21 Probe specificity was verified by evaluating positive (eg, slide prepared from cultured H. pylori) and negative (slide prepared from cultured Escherichia coli) control slides in each assay. The gastric bacteria were visualized with an Olympus BX51® epifluorescence microscope and photographs were acquired with an Olympus DP-7 camera. Quantification of mucosal bacteria was performed in a semiquantitative fashion with the investigators (MB, KWS) blinded to the infection status of the animal. Grading (+ to ++++) reflected the highest amount of helicobacter colonization observed in a tissue section at ×60 magnification.

Deparaffinization and DNA Extraction

Two 20 μm sections of each tissue block were deparaffinized according to the manufacturer’s instructions. Briefly, samples were immersed in 1,200 μL xylene, vortexed, and centrifuged for 5 minutes at 12,000 rpm. The supernatant was removed and 1,200 μL ethanol added to the pellet. The samples were vortexed and centrifuged again after which time the supernatant was discarded. After a 2nd wash with ethanol, the pellets were air-dried at 37°C for 15 minutes. DNA extraction was performed as recommended and

Table 1. Probes and hybridization conditions for fluorescence in situ hybridization.

<table>
<thead>
<tr>
<th>Probe</th>
<th>Sequence 5' → 3'</th>
<th>Target</th>
<th>Hybridization Conditions</th>
<th>Washing Conditions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eub</td>
<td>GCT GCC TCC CGT AGG AGT</td>
<td>Eubacteria</td>
<td>As used for specific probe 20 mM Tris, 0.9 M NaCl, 0.1% SDS</td>
<td>40% formamide pH 7.2 46°C overnight</td>
<td>Amann et al21</td>
</tr>
<tr>
<td>Hel 274</td>
<td>GGC CGG ATA CCC GTC ATW GCC T</td>
<td>Helicobacter spp.</td>
<td>As used for specific probe 20 mM Tris, 0.9 M NaCl, pH 7.2</td>
<td>48°C for 20 minutes</td>
<td>Chan et al22</td>
</tr>
<tr>
<td>Hel 717</td>
<td>AGG TCG CCT TCG CAA TGA GTA</td>
<td>Helicobacter spp.</td>
<td>As used for specific probe 20 mM Tris, 0.9 M NaCl, pH 7.2</td>
<td>48°C for 20 minutes</td>
<td>Chan et al22</td>
</tr>
</tbody>
</table>

SDS, sodium dodecyl sulfate.

FISH and Helicobacter-Associated Gastritis
DNA was eluted 2 times in 100μL of elution buffer yielding 200μL in total. DNA concentration was measured with a Biophotometer spectrophotometer. Negative controls were included in each deparaffinization and DNA extraction run to evaluate for contamination.

**PCR Analysis**

Primers for dog β-actin 131 forward (5′-CAC CCT GAA GTA CCC CAT TGA G) and dog β-actin 211 reverse (5′-TTG TAG AAG GTG TGG TGC CAG AT), which yield an amplicon of approximately 81 base pairs (bp) with genomic DNA, were used to evaluate the integrity of the DNA extracted from archival tissue blocks.4 PCR was performed in an Eppendorf thermocycler with Promega Green Master Mix and visualized with ethidium bromide. The size of the expected fragments (398 and 251 and 78 bp) was compared with a 100-bp reference marker.

For *Helicobacter* PCR, primers that produced amplicons ≤ 400 bp were chosen to enable optimal detection in archival formalin-fixed, paraffin-embedded tissues.23 Primers C97 forward (5′-GCT ATG ACG GGT ATC C) and C98 reverse (5′-GAT TTT ACC CCT ACA CCA) were used to amplify a 398-bp fragment of the 16S rRNA gene. All PCR products obtained with the C97/C98 PCR additionally were amplified with an internal pair of primers (HelF, forward: 5′-CGT GGA GGA TGA AGG TTT TA-3′, location within the 398-bp fragment 128–147; HelR2, reverse: 5′-AAT TCC ACC TAC CTC TCC C-3′, location within the 398-bp fragment 360–379) producing a 251-bp fragment.17 PCR was performed in an Eppendorf thermocycler4 with Promega Green Master Mix (MgCl₂ concentration 1.5 mM), 0.2 mM of each primer, in a reaction volume of 24μL, and 1μL of target DNA. The C97/98 cycle was denaturated at 94 °C for 5 minutes, 94 °C for 30 seconds, 58 °C for 30 seconds, 72 °C for 30 seconds (40 cycles), with a final extension at 72 °C for 7 minutes. For nested PCR, 2μL of the 1st reaction was diluted 1:8 in ultrapure distilled water, and used as template DNA, with identical cycling conditions for 35 cycles. Negative controls in which DNA extract was replaced by sterile distilled water were included with each reaction. DNA extracted from a dog stomach sample positive for Helicobacter spp, was used as a positive control. PCR products were subjected to agarose gel electrophoresis and visualized with ethidium bromide. The size of the expected fragments (398 and 251 and 78 bp) was compared with a 100-bp reference marker.

**Results**

Three dogs and 2 cats with *Helicobacter*-associated gastritis had signs of persistent, nonspecific gastroenteritis (Table 2). Vomiting of bile-stained ingesta was the salient sign in all animals. Routine diagnostic evaluation often was unremarkable but 1 cat had minor increases in hepatic enzyme (alanine aminotransferase, alkaline phosphatase) activities on initial presentation. Supplemental diagnostic tests (eg, abdominal ultrasonography [n = 2], assays for feline pancreatic lipase immunoreactivity [n = 2], and serum folate and cobalamin concentrations [n = 2]) performed in some animals failed to identify abnormalities. Gastrointestinal endoscopy indicated erosions and increased mucosal friability in the stomachs of dogs and cats with gastritis. Mucosal erosions generally were localized to the gastric body and antral regions. Cytologic examination detected abundant gastric spiral organisms in all animals at diagnosis. Mucosal histopathology indicated gastritis of varying (eg, moderate to severe lymphocytic-plasmacytic gastritis [n = 2]; mild to severe lymphocytic gastritis [n = 3]); severity characterized by lymphonodular mucosal hyperplasia, mononuclear cellular inflammation, architectural (glandular) distortion (eg, atrophy and fibrosis), and mucosal spirochetes (Fig 1). Mildly increased numbers of lymphocytes and plasma cells were observed in the duodenal lamina propria of 1 dog (9-year-old neutered male Beagle).

**Table 2.** Clinicopathologic summary of *Helicobacter*-associated gastritis in dogs and cats.

<table>
<thead>
<tr>
<th>Signalment</th>
<th>9 year MC Beagle</th>
<th>2.5 year F Vizsla</th>
<th>3 year MC Terrier</th>
<th>8 year MC DSH</th>
<th>1 yr FS DSH</th>
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<tbody>
<tr>
<td>GI signs</td>
<td></td>
<td></td>
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<tr>
<td>Pretreatment</td>
<td>Vomiting/diarrhea for 2 months</td>
<td>Vomiting for 9 months</td>
<td>Vomiting for 1 month</td>
<td>Vomiting for 3 months</td>
<td>Vomiting/diarrhea for 4 months</td>
</tr>
<tr>
<td>Posttreatment</td>
<td>Signs resolved</td>
<td>Signs resolved</td>
<td>Signs resolved</td>
<td>Signs resolved</td>
<td>SB diarrhea</td>
</tr>
<tr>
<td>Gastric cytology</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Pretreatment</td>
<td>Moderate GSOs</td>
<td>Abundant GSOs</td>
<td>Moderate GSOs</td>
<td>Abundant GSOs</td>
<td>NA</td>
</tr>
<tr>
<td>Posttreatment</td>
<td>No GSOs</td>
<td>No GSOs</td>
<td>No GSOs</td>
<td>No GSOs</td>
<td>No GSOs</td>
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<tr>
<td>Histopathology</td>
<td></td>
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<tr>
<td>Pretreatment</td>
<td>L gastritis</td>
<td>L gastritis</td>
<td>LP gastritis</td>
<td>LP gastritis</td>
<td>LP gastritis</td>
</tr>
<tr>
<td>Posttreatment</td>
<td>Intraglandular GSOs</td>
<td>Intraglandular GSOs</td>
<td>Intraglandular GSOs</td>
<td>Intraglandular GSOs</td>
<td>Intraglandular GSOs</td>
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<td>FISH analysis</td>
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<tr>
<td>Pretreatment</td>
<td>Positive +</td>
<td>Positive +++</td>
<td>Positive +++</td>
<td>Positive +++</td>
<td>Positive +++</td>
</tr>
<tr>
<td>Posttreatment</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
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<tr>
<td>PCR analysis</td>
<td></td>
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<tr>
<td>Pretreatment</td>
<td>Negative</td>
<td>Positive 250 bp</td>
<td>Positive 250 bp, 400bp</td>
<td>Positive 250 bp</td>
<td>Positive 250bp, 400bp</td>
</tr>
<tr>
<td>Posttreatment</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

GI, gastrointestinal; MC, male castrate; F, female intact; FS, female spayed; GSOs, gastric spiral organisms; L, lymphocytic; LP, lymphocytic-plasmacytic; NA, not available; FISH, fluorescence in situ hybridization; PCR, polymerase chain reaction.
Analysis of gastric endoscopic specimens by FISH confirmed moderate numbers of mucosally associated helicobacters in 2/3 dogs and both cats before drug therapy (Fig 2). The remaining dog had only sparse (2–4 bacteria/section) colonization, but tissue hybridization with *Helicobacteraceae*-specific probes labeled with Cy-3 identified these organisms by their fluorescence and unique spiral morphology. Genus-specific PCR was positive in pretreatment archival tissue blocks from 4/5 animals (Table 2): Two of 5 samples yielded the expected 400-bp amplicon with primers C97/C98 whereas the more sensitive nested PCR, with primer set HelF/HelR2, yielded the expected 250-bp amplicon in 4/5 animals (Table 2). Rapid resolution of vomiting episodes (within 7 days) was observed in all animals after elimination diet therapy and triple therapy with amoxicillin, metronidazole, and bismuth subsalicylate. Small intestinal diarrhea persisted in 1 cat despite resolution of vomiting episodes. Long-term outcome determined by repeat examinations and telecommunication indicated that the original signs did not recur in 4/5 animals for up to 38 months (eg, Beagle—18 months, Vizsla—38 months, Terrier—9 months, 8-year-old domestic shorthair cat—36 months) of follow-up. The remaining cat failed to return for repeat clinical evaluation after the 2nd endoscopic examination. Posttreatment gastric biopsies collected 4–14 weeks after therapy and examined with H&E and Warthin-Starry stains failed to reveal the presence of spiral bacteria in mucosal tissues from any of the 5 animals. The severity of gastric inflammation did not differ appreciably between biopsies obtained before and after triple drug therapy. The cat with persistent diarrhea was subsequently diagnosed with mild lymphocytic-plasmacytic enteritis and responded to an elimination diet and prednisolone (1 mg/kg PO q12h for 2 weeks then tapered) therapy. Intestinal biopsies from the remaining animals revealed no morphologic abnormalities. Repeat FISH analysis performed posttherapy confirmed clearance of visible gastric *Helicobacter* spp. organisms in all animals. PCR amplification of posttreatment gastric samples with *Helicobacter* genus-specific primers was negative in all animals. Pre- and posttreatment gastric DNA amplified with β-actin primers yielded the expected 81-bp amplicon in each sample.

**Discussion**

The cause of gastritis in dogs and cats is seldom determined, but systemic disease, ulcerogenic drugs, and host immune responses to parasites, dietary antigens, or bacterial components all may cause mucosal inflammation. *Helicobacter* spp. infection has been associated with gastric disease in diverse species including humans, ferrets, cheetahs, pigs, and laboratory animals. To date, numerous gastric *Helicobacter* spp. have been reported in populations of dogs and cats, including random-source animals, clinically healthy animals, laboratory Beagles, and shelter dogs. Although *Helicobacter* organisms frequently are demonstrated in gastric biopsy specimens obtained from sick client-owned dogs and cats, their role in gastritis as a potential pathogen has not been clearly established.
The present report documents the resolution of clinical signs after clearance of visible gastric Helicobacteriaeae, which may have caused or contributed to chronic vomiting and gastritis in some dogs and cats. However, a direct causal relationship between Helicobacter spp., clinical signs, and gastritis could not be established. Clinical signs of small intestinal diarrhea did not completely resolve in the 2nd cat, but medical therapy for enteritis was curative. Special stains for Helicobacter spp. and other infectious causes for mucosal inflammation in intestinal biopsies of this cat were negative. It is noteworthy that mild-to-moderate duodentitis has been reported previously in dogs with chronic vomiting and concurrent gastric Helicobacter spp. A diagnosis of Helicobacter-associated gastric disease (eg, gastritis) was suspected in the animals of the present study on the basis of their gastrointestinal signs and histopathologic findings of mucosal cellular infiltrates that often formed lymphoid nodules, intraglandular spiral bacteria, and architectural distortion. Interestingly, these changes were readily apparent in H&E-stained sections and equally prevalent between biopsy specimens of fundic and antral mucosa.

Two animals diagnosed with helicobacter-associated gastritis had histories of small bowel diarrhea alone (cat) or in association with chronic vomiting (dog). A review of the cat’s history revealed that this animal was indeed vomiting, but it was originally suspected that these episodes were associated with trichobezoars despite the fact that emesis consisted only of bile-stained ingesta. Thus, it remains possible that these intermittent vomiting episodes were owing to gastritis. Pretreatment duodenal histopathology in this cat was normal and provided no insight as to the etiopathogenesis for diarrhea. However, repeat enteroscopy with duodenal biopsy confirmed the presence of mild, chronic lymphocytic-plasmacytic enteritis, which readily responded to dietary and pharmacologic therapy. The exact cause(s) for the small bowel diarrhea noted in the Beagle at presentation remains unknown. It is unlikely that this dog had clinically relevant inflammatory enteropathy owing to absence of objective indices of mucosal inflammation or enterocyte injury observed on review of duodenal biopsy specimens. We hypothesize that this dog likely had a food-responsive chronic enteropath as evidenced by the mild diarrhea, subtle increase in mucosal lymphocytes and plasma cells on histology, and complete resolution of diarrhea when treated with an elimination diet concurrent with triple therapy.

Several diagnostic tests, including exfoliative cytology, histopathology, and the rapid urease test, have been shown to be accurate indices for diagnosing infection with gastric helicobacters in dogs and cats, with some limitations. CYtologic specimens may not detect the presence of concurrent mucosal inflammation or lymphoid follicular hyperplasia, and the type and severity of gastritis reveals considerable overlap between infected and uninfected dogs. A more consistent correlation between Helicobacter spp. infection and the extent of gastritis in cats recently has been demonstrated. However, Helicobacter are not always recognized on routine H&E-stained tissue sections, and specific histochemical staining methods (eg, Warthin-Starry, Steiner stains) are less sensitive than PCR and gastric cytology for detecting gastric Helicobacter-like organisms. The modified Steiner stain is a nonspecific silver stain that stains many bacteria other than Helicobacter as well as argyrophilic cellular inclusions that may be mistaken for bacteria. Argyrophilic or argentaffin neuroendocrine cells of the gastrointestinal tract stain positive and granules within these cells can be confused with bacteria. This is a particular problem in dogs and cats where approximately 10% of Helicobacter-like organisms are intracellular. Cytomegalovirus-infected cells in the stomachs of humans also have been reported to stain positive with argyrophilic stains which confounds accurate diagnosis of gastric intracellular bacteria. Finally, detection of gastric urease, the basis of the urea breath test and biopsy urease test, is less sensitive than histochemical staining and can be nonspecific owing to urease production by non-Helicobacter species.

FISH offers greater specificity than immunohistochemical stains and permits visualization of Helicobacter spp., in contrast to PCR, which only detects the presence of the organism. As proof of concept of the superiority of FISH in detecting Helicobacter infection, we observed low numbers (+) of fluorescent bacteria in 1 dog’s pretreatment biopsies that were negative by PCR with 2 sets of Helicobacter genus-specific primers. We attributed the discordance between FISH and PCR to likely reflect the low bacterial numbers and patchy distribution of mucosal helicobacters in this dog. Thus, in the present study, oligonucleotide probes specific to the 16S rDNA gene sequence of Helicobacteriaeae permitted diagnosis of Helicobacter-associated gastritis in formalin-fixed paraffin-embedded biopsy specimens. These probes were designed to target the family Helicobacteriaeae, that includes both Wolinella spp. and Helicobacter spp. However, Wolinella spp. is a very restricted subset of bacteria (eg, a small proportion of the Epsilon Proteobacteria and Campylobacteria) and is much more restrictive than other bacteria, such as Bartonella, Leptospira, Borrelia, and E. coli, which are also targeted by argyrophilic stains. In addition, we are unaware of any studies that have demonstrated Wolinella spp. organisms in the stomachs of dogs or cats. It, therefore, seems likely that the molecular probes utilized in this study accurately identified Helicobacter spp. localized within gastric mucosa before triple therapy and confirmed successful clearance of visible gastric Helicobacters after treatment in all animals.

The criteria for eradication used in Helicobacter-infected human patients are negative urease breath tests, negative gastric urease activity, or negative histopathology with Giemsa or silver stains such as Steiner or Warthin-Starrry. The eradication of H. pylori in symptomatic humans has been associated with the attenuation of both clinical signs and gastritis. Triple therapy with a proton-pump inhibitor with clarithromycin and amoxicillin or metronidazole given twice daily remains the recommended 1st choice treatment. Bismuth-containing quadruple therapy, if available, is also a 1st choice treatment option. Well defined guidelines for treating dogs
and cats with gastric Helicobacter infection have not been formulated. The accumulated data to date suggest that combination therapy with antimicrobials (eg, amoxicillin, metronidazole) and acid secretory inhibitors (eg, famotidine, omeprazole) may not eradicate Helicobacter spp. in all dogs and cats. For example, triple therapy with or without famotidine was equally effective in decreasing clinical signs of gastritis in dogs 6 months posttreatment but eradicated gastric bacteria in only 40% of the dogs. Similarly, Happonen et al revealed that triple therapy for 14 days eradicated gastric helicobacters in 7/9 dogs, but that gastric helicobacters recurred in 4/4 dogs within 3 years of eradication treatment. Whether antibiotic failure was attributable to reexposure or recrudescence in these earlier reports was unclear. Studies have identified gastric Helicobacter spp. in diverse extragastric locations, including the oral cavity (eg, saliva, dental plaque, or gingival pockets), where protection from antimicrobial therapy might facilitate gastric recolonization and recrudescence of infection. Reinfection via the fecal-oral route has been postulated to occur in dogs owing to their social behavior. In contrast to these other reports evaluating Helicobacter spp. status, a pilot study in dogs revealed an 80% eradication rate for up to 30 days after 1 week of triple therapy with omeprazole, metronidazole, and spiramycin. Although the results of the present study indicated clearance of gastric helicobacters for up to 14 weeks after therapy, it is possible that some Helicobacter spp. in dogs and cats would have been identified if all animals had been tested at time points later than 14 weeks after completing therapy. Triple therapy administered for 21 days resulted in rapid clinical improvement and the clearance of visible Helicobacter spp. infection in the dogs and cats of this report. Thorough diagnostic evaluation by cytologic, histologic (H&E and Warthin-Starry stains), and FISH analysis of gastric biopsy specimens proved that these animals were negative for Helicobacter spp. for up to at least 14 weeks after treatment. Furthermore, we confirmed successful clearance (eg, eradication) of gastric helicobacters in these animals by use of a sensitive Helicobacter genus-specific PCR technique which recently has been validated for use in dogs and cats. Long-term follow-up was available in 3 dogs and 1 cat of the present study and revealed all animals to be free of clinical signs of gastritis. Posttreatment remission times ranged from 9 to 38 months. The 1 cat originally diagnosed with enteritis has been placed on a hydrolyzed protein diet and requires intermittent low-dose glucocorticoid therapy for occasional disease flares.

The observation of gastric inflammation that remained unchanged after clearance of Helicobacter spp. infection in the dogs and cats of this study is similar to that reported by others. Our data indicate that chronic gastritis still was present in gastric mucosal biopsies 14 weeks after cessation of triple therapy. In a prospective study of helicobacter-positive pet dogs, Happonen et al revealed that neither triple therapy nor additional therapies had a significant effect on severity of gastric histologic changes when followed long term (up to 3 years) in 4 dogs. Furthermore, other studies have shown that there is no apparent association between Helicobacter spp. infection and severity of gastritis in dogs, and that histologically verified chronic gastritis may be seen in healthy dogs. In contrast, Madisch et al has observed that humans have complete and long-lasting (as early as 3 months and persisting up to 12 months after treatment) healing of lymphocytic gastritis after H. pylori eradication with triple therapy. Leib et al also recently revealed that eradication of gastric Helicobacter spp. was associated with improvement in gastritis scores, especially 6 months after dogs received either triple or quadruple therapy. Whether complete healing of lymphocytic gastritis after clearance of Helicobacter spp. might have occurred over time in the dogs and cats of this study remains undetermined.

There are several limitations in this study that warrant further consideration. We cannot be certain that resolution of vomiting episodes was owing to triple therapy alone versus triple therapy combined with an elimination diet. It was our intent to use the accepted “gold standard” for treatment of humans and dogs infected with Helicobacter spp. Treatment iterations of triple therapy or the use of a placebo group were not performed because (1) this approach would have been contrary to best practice, evidence-based recommendations for treatment of Helicobacter-associated gastritis and (2) use of a placebo group was deemed impractical because animals may have continued to exhibit clinical signs and clients would have been intolerant of this negative treatment response. The long-term follow up (eg, 9–38 months) in 4/5 animals that revealed persistent resolution of gastrointestinal signs argues against the idea that reduction in severity of gastrointestinal signs was owing to normal variation of the disease process itself.

To our knowledge, the role of diet in treatment of Helicobacter spp. in dogs or cats has not been reported. The use of elimination diets as adjuvant therapy in most animals with chronic gastroenteritis is standard therapy at our institution. We note that Leib et al maintained Helicobacter-infected dogs on their current diet during drug therapy in an earlier study, but the nature of this diet (eg, commercial adult diet, highly digestible controlled diet, intact protein elimination diet, hydrolysate elimination diet) was not disclosed. Some dogs with chronic vomiting and Helicobacter spp. might have been fed an elimination diet in conjunction with drug therapy for gastritis.

In conclusion, the present study indicates that Helicobacter spp. infection in some dogs and cats may be associated with clinical signs and histopathologic lesions of chronic gastritis. FISH with 16S rDNA-targeted oligonucleotide probes applied to gastric biopsy specimens provides a sensitive and specific method for detection of Helicobacter spp. infection. These probes are commercially available, the method is cost-effective, and FISH can be applied in diverse laboratory settings without the need for specialized equipment, with the exception of a fluorescence microscope. This technique should be particularly useful for confirmation of infection in cases equivocal by other methods, and potentially useful in posttreatment evaluation. Although
triple therapy with amoxicillin, metronidazole, and bis-muth subsalicylate was successful in the resolution of chronic signs and clearance of gastric Helicobacter spp. for up to 7 weeks after treatment, gastric inflammation persisted in these animals.

Footnotes


