Enterohepatic *Helicobacter* spp. in cats with non-haematopoietic intestinal carcinoma: a survey of 55 cases

Alton G. Swennes,1† Nicola M. A. Parry,1 Yan Feng,1 Erin Sawyer,1‡ Bryan R. Lohr,2 David C. Twedt3 and James G. Fox1

1Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA, USA
2Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Purdue University, West Lafayette, IN, USA
3Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, USA

Several enterohepatic *Helicobacter* spp. (EHS) have been isolated from cats. Despite the reported association between EHS infection and intestinal neoplasia in other species, this association has not been explored in cats. In this study, 55 non-haematopoietic feline intestinal carcinoma cases were histopathologically evaluated. In contrast with prior reports, large intestinal (LI) carcinoma was observed with greater frequency (61 %) relative to small intestinal (SI) carcinoma (35 %). There was a significant association between intestinal location and animal gender. Of males examined, 83 % had LI carcinoma, while no such trend was observed in females. Previously described associations between Siamese breed and intestinal carcinoma could not be definitively confirmed, although the Siamese breed may be predisposed to SI carcinoma location. Of all carcinomas examined in this study, 62 % were classified as adenocarcinoma, although mucinous adenocarcinoma (28 %) and solid carcinoma (11 %) were also identified. Tumours were all moderately or poorly differentiated. When considered by intestinal location and histopathologic classification, LI adenocarcinoma was associated with significantly advanced mean age (13 years) when compared to SI adenocarcinoma and LI mucinous adenocarcinoma (mean, 9 years in both cases), which were also frequently encountered. To determine whether EHS might play a role in feline intestinal neoplasia, *Helicobacter* genus- and species-specific fluorescence *in situ* hybridization was performed. Of these carcinoma cases, 56 % were positive for *Helicobacter* spp. and one or more species-specific assay for *Helicobacter bilis*, *Helicobacter canis* or *Helicobacter marmotae*. The presence of EHS was significantly associated with both LI location (68 %) and mucinous adenocarcinoma (92 %). These findings suggest a role for intestinal bacteria in non-haematopoietic feline intestinal neoplasia.

INTRODUCTION

Enterohepatic *Helicobacter* spp. (EHS) colonize the hepatobiliary systems and intestinal tracts of many species, producing host-dependent diarrhoea, inflammation and neoplasia (Whary & Fox, 2004). In susceptible mouse strains, the model EHS *Helicobacter hepaticus* acts as a microbial trigger promoting typhlocolitis and intestinal neoplasia (Fox et al., 2011; Nagamine et al., 2008; Li et al., 1998; Fox et al., 1996b). In cats, a culture-based survey
identified a greater than 9% EHS infection prevalence among 227 research cats (Shen et al., 2001). 16S rRNA gene sequencing of these strains identified *Helicobacter canis*, *Helicobacter bilis* and *Helicobacter marmotae*. Another survey of 84 pet cats found a 21% EHS prevalence, and *H. canis*, *H. bilis* and *Helicobacter cinaedi* were isolated from both diarrheic and non-diarrheic cats (Rossi et al., 2008). Thus, several EHS are prevalent in cats, although their precise role in disease remains incompletely characterized.

These species have been associated with a variety of disease presentations in dogs, cats and humans. *H. canis* has been identified in Bengal cats with diarrhoea (Foley et al., 1999), dogs with hepatitis (Fox et al., 1996a) and diarrhoea (Castiglioni et al., 2012; Stanley et al., 1993) and humans with gastroenteritis (Burnens et al., 1993), autoimmune hepatitis (Casswall et al., 2010), inflammatory bowel disease (Tankovic et al., 2011) and bacteriemia (Abidi et al., 2013; Prag et al., 2007; Leemann et al., 2006; Gerrard et al., 2001). *H. bilis* has been associated with human chronic cholecystitis and biliary cancer (Matsukura et al., 2002; Fox et al., 1998) and with inflammatory bowel disease in mice (Maggio-Price et al., 2006). *H. marmotae* has been identified in woodchuck livers and cat faeces, although its association with disease is incompletely characterized (Fox et al., 2002; Patterson et al., 2010; Beisele et al., 2011). Isolation of these species from companion animals and from humans has fueled speculation of inter-species transmission. Natural EHS infection has also been associated with colitis and intestinal adenocarcinoma in non-human primates. In rheus macaques, EHS were commonly cultured in diarrhoea with a 57% prevalence in a non-diarrheic cohort housed in different institutions (Fox et al., 2007, 2001). Subsequent studies have identified persistent infection and 93% EHS prevalence among rheus macaques with intestinal adenocarcinoma (IAC) (Marini et al., 2010; Lertpiriyapong et al., 2014). EHS have similarly been isolated from cotton-top tamarins, another colitis- and IAC-prone species (Saunders et al., 1999).

Despite the association between EHS infection and neoplasia and prior EHS isolation from cats, EHS infection has not been studied in the context of non-haematopoietic feline intestinal neoplasia. In this study, 55 feline intestinal carcinoma cases were retrospectively evaluated. Associations between intestinal tumour location and histopathologic type were identified based on patient sex, breed and age. To address the possible association between EHS infection and neoplasia, *Helicobacter* spp. infection status was determined using fluorescence in situ hybridization (FISH). While EHS were commonly identified in carcinoma-positive and carcinoma-negative cats, EHS infection was significantly associated with the development of poorly differentiated large intestinal (LI) mucinous adenocarcinoma. Thus, it is possible that EHS play a role in intestinal carcinoma formation and topology in cats.

**METHODS**

**Study design.** This study retrospectively examined 55 feline intestinal carcinoma cases diagnosed during 1997–2006 at a veterinary school hospital by veterinary pathologists after surgical resection or necropsy. The group contained 31 females and 24 males. Sixteen cats were Siamese, one was a Maine Coon and 38 were random bred. Their mean age was 12 years range, 4–18), excluding three of unreported age. A separate group, composed of 22 cats euthanized at an animal shelter for reasons unrelated to gastrointestinal disease, was utilized to examine feline EHS prevalence. Their mean age was 6 years (range, 2 months–14 years), excluding three of unreported age.

**Histopathology.** Intestinal samples were fixed in 10% neutral buffered formalin, embedded in paraffin wax, cut into 4-μm sections and mounted on glass slides. Sections were stained with hematoxylin and eosin or Periodic acid-Schiff for diagnostic evaluation by a board-certified veterinary pathologist (N. M. A. P.). Upon microscopic evaluation, tumours were classified based on histopathologic characteristics into one of three categories. Solid carcinoma was composed of undifferentiated neoplastic cellular sheets, with no mucin or gland formation. Cells were frequently pleomorphic and round to polygonal with eosinophilic cytoplasm and had a round, variably sized nucleus with prominent nucleoli. Up to 10 mitotic figures were present per 10 high-power fields. Adenocarcinoma comprised glands forming acini or tubules lined by cuboidal to columnar neoplastic cells with eosinophilic cytoplasm and a round, variably sized nucleus with prominent nucleoli. Up to four mitotic figures were present per 10 high-power fields. Some neoplastic glands were mucin expanded. *Mucinous adenocarcinoma* describes those tumours in which mucin represented more than 50% of the tumour. These were predominantly composed of acini and tubules, but solid carcinomatous features were also present. Up to seven were present per 10 high-power fields. Moderate to large quantities of mucin expanded neoplastic glandular structures and frequently formed indiscrte lakes that dissected the submucosal and muscularis layers and were typically surrounded by isolated signet-ring-shaped cells.

**Statistical analysis.** For analysis of nominal variables, group-wise comparisons were performed using Fisher’s exact test. For analysis of continuous variables (patient age), data were tested for normality using the D’Agostino–Pearson omnibus normality test. Because data were normally distributed, a one-way ANOVA with Tukey’s multiple comparison procedure was used. Calculations were performed using GraphPad PRISM 6 (GraphPad Software); α=0.05.

**Fluorescent oligonucleotide probes.** Fluorescent oligonucleotide probes were either used as previously described (Chan et al., 2005) or designed using DNASTAR (Table S1, available in the online Supplementary Material). Probes were synthesized by Integrated DNA Technologies and labeled with Cy3. Probes were tested against positive controls; both glass slides were spotted with cultured bacteria and paraffin-embedded tissues from mouse experimental infections. All probes were tested against *Helicobacter pylori* ATCC 26695 and SS1, *Helicobacter felis* ATCC 49179, *H. hepaticus* ATCC 51449, *H. canis* ATCC 51402, *Helicobacter rodentium* MIT 95-1707, *Helicobacter cinaedi* ATCC 13284, *H. bilis* ATCC 51630, *H. marmotae* ATCC 700968, *Helicobacter pullorum* ATCC 700968, *Helicobacter cholecysticus* ATCC 700242, *Helicobacter rupini* and *Campylobacter jejuni* 81-176. Probes were further evaluated using gastric or intestinal sections from mice infected with *H. pylori*, *H. hepaticus*, *H. bilis*, *H. marmotae*, *H. cinaedi*, *Helicobacter trorgenium* and *H. rodentium*. Infection status of these mice was confirmed by PCR of non-fixed intestinal tissue or faeces.

**Fluorescence in situ hybridization.** FISH was performed as previously described (Bridgeford et al., 2008). For probe validation, bacteria were collected from blood agar plates, fixed with 4% paraformaldehyde,

http://jmm.microbiologyresearch.org
spotted onto slides and air dried. For paraffin-embedded tissue, unstained sections were deparaffinized by passage through xylene (three times for 10 min), 100% ethanol (three times for 2 min) and 95% ethanol (three times for 2 min) and air dried. FISH probes were reconstituted with sterile water and diluted to 10 ng µl⁻¹ with hybridization buffer (20 mM Tris-HCl, 0.9 M sodium chloride, 0.1% SDS, 30% formamide, pH 7.2). Fifty microliters of probe solution was applied to slides. Sections were covered in parafilm to minimize evaporation and placed in a hybridization chamber at 48°C overnight. Slides were washed with 2 buffers (buffer 1: 20 mM Tris-HCl, 0.9 M NaCl, 0.01% SDS; buffer 2: 20 mM Tris-HCl, 0.9 M NaCl) at 48°C for 15 min each, rinsed with water, air dried and mounted in VECTASHIELD HardSet mounting medium with DAPI (Vector Laboratories).

RESULTS

Histopathology

Intestinal carcinoma segmental location was categorized as small intestinal (SI) or LI. Several locations were not precisely described and could not be determined retrospectively owing to lack of normal microscopic anatomy. Of those categorized, 31/51 (61%) were LI, including one that was colorectal, and slightly fewer (18/51, 35%) were SI (Table 1). There was significant association between intestinal location and patient sex (P=0.016). In males, 19/23 (83%) cases had LI carcinoma. In females, SI and LI carcinomas were encountered in nearly equal numbers, with females thus accounting for 14/18 (78%) SI cases. Because relatively few intact male (6/24, 25%) and intact female (5/31, 16%) cats were included in the case cohort, the impact of neutered status on tumour formation could not be definitively determined. However, removal of these cases did not substantially alter the results. Two cases not included in the above totals had large masses involving multiple structures at the ileocecal (ICC) junction, although additional LI caecal and proximal colonic (n=3) as well as SI terminal ileal (n=2) carcinomas could also be categorized as involving the ICC junction. Thus, 7/51 (14%) tumours involved structures of the ICC junction.

In cases where tumour sections were available (n=47), they were classified as solid carcinoma, adenocarcinoma or mucinous adenocarcinoma based on their predominant histopathologic characteristics (Fig. 1; see Methods). Most cases (29/47, 62%) were classified as adenocarcinoma, although mucinous adenocarcinoma (13/47, 28%) and solid carcinoma (5/47, 11%) were also noted. Most tumours were highly malignant, and evidence of metastasis was observed in 49/55 (89%) cases, with peritoneal carcinomatosis being frequently identified (43/55, 78%). Variable neoplastic extension into subjacent submucosal, muscularis and serosal layers was noted in all categories. Desmoplasia

Table 1. Patient characteristics (age, sex and proportion belonging to the Siamese breed) shown based on intestinal tumour location and based on both intestinal tumour location and histopathologic characteristics

<table>
<thead>
<tr>
<th>Intestinal carcinoma, all types</th>
<th>Location</th>
<th>Small intestine</th>
<th>Large intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td></td>
<td>18</td>
<td>31</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td></td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>8/18 (44%)‡</td>
<td>19/31 (61%)‡</td>
</tr>
<tr>
<td>Siamese</td>
<td></td>
<td>8/18 (44%)†</td>
<td>6/31 (19%)†</td>
</tr>
</tbody>
</table>

Intestinal carcinoma cases separated by histopathologic characteristics

| Solid carcinoma               | n         | 4‡               | 1‡             |
| Mean age (years)              |          | 12               | 12             |
| Male                          |          | 2/4 (50%)        | 1              |
| Siamese                       |          | 3/4 (75%)        | 0              |

Adenocarcinoma

| n                              |          | 9‡               | 18‡            |
| Mean age (years)               |          | 9§               | 13§,#          |
| Male                          |          | 2/9 (22%)        | 11/18 (61%)    |
| Siamese                       |          | 3/9 (33%)        | 6/18 (33%)     |

Mucinous adenocarcinoma

| n                              |          | 2‡               | 10‡            |
| Mean age (years)               |          | 15               | 9§             |
| Male                          |          | 0/2 (0%)         | 6/10 (60%)     |
| Siamese                       |          | 2/2 (100%)       | 0/10 (0%)      |

Undetermined

| n                              |          | 3                | 2              |
| Mean age (years)               |          | 8                | 15             |
| Male                          |          | 0/3 (0%)         | 2/2 (100%)     |
| Siamese                       |          | 0/3 (0%)         | 0/2 (0%)       |
was observed in 42/47 (89%) cases, particularly in those involving mural extension. Lymph node metastasis was variably noted, although appropriate sections were often not available. Concurrent lymphoma was evident in 4/55 (7.2%) individuals. There was no evidence of papillary epithelial formation, osseous metaplasia or cartilaginous metaplasia in any cases.

Carcinoma intestinal location and histopathologic characteristics were considered together when both were definitively known (n=44; Table 1). There was a significant association between intestinal location and histopathologic type (P=0.044). The most common location category was LI adenocarcinoma (18/44, 41%), with SI adenocarcinoma (9/44, 20%) and LI mucinous adenocarcinoma (10/44, 23%) also being frequently noted. Interestingly, cats with LI adenocarcinoma (mean age, 13 years) were significantly older than those with SI adenocarcinoma (mean age, 9 years; P=0.012) or LI mucinous adenocarcinoma (mean age, 9 years; P=0.020). All other categories (7/44, 16%) were relatively uncommon, with one to four cases encountered.

Tumours were classified based on glandular differentiation and were classified as moderately differentiated if glandular structures occupied 50–95% of tumour area or poorly differentiated if composed of less than 50% glandular structures. No well-differentiated tumours were observed. LI adenocarcinomas were either moderately (11/17, 65%) or poorly (6/17, 35%) differentiated, with a 44% (range, 5–65%) glandular composition. SI adenocarcinomas were also either moderately (2/9, 22%) or poorly (7/9, 78%) differentiated, with a mean of 28% (range, 10–60%) tumour area. All LI mucinous adenocarcinomas were poorly differentiated and had 44% (range, 20–55%) glandular composition. Tumours in remaining categories were poorly differentiated; no well-differentiated tumours were noted.

Prior studies have suggested a higher intestinal carcinoma incidence in the Siamese breed (Kosovsky et al., 1988; Patnaik et al., 1976; Turk et al., 1981; Rissetto et al., 2011). While this study could not determine incidence, Siamese carcinoma cases (n=14) were analysed in terms of segmental distribution and histopathologic category where these were known (Table 1). There was a borderline statistical relationship between Siamese breed and SI tumour location (P=0.056). Siamese cats accounted for 8/18 (44%) SI carcinomas despite being only 14/51 (27%) cases of known intestinal location. In addition, 3/4 (75%) SI solid carcinomas and 2/2 (100%) SI mucinous adenocarcinomas occurred in Siamese cats.

**Helicobacter spp. detection**

To examine the associations between feline intestinal carcinoma and EHS infection, *Helicobacter* genus- and species-specific FISH was performed on intestinal sections (Table 2, Fig. 1j). EHS were prevalent in cats with intestinal carcinoma (30/54, 56%). Given the trends noted among carcinoma cases regarding intestinal location, histopathologic category, age, sex and breed, FISH results were evaluated in these contexts (Table 2). EHS infection was noted in 21/31 (68%) LI tumour cases, but only in 6/18 (33%) SI tumour
cases (P=0.036). *H. bilis* infection was also significantly more common in LI tumour cases (P=0.018). Importantly, EHS infection was very common (12/13, 92 %) among cats with mucinous adenocarcinoma compared to other histopathologic categories (16/33, 48 %; P=0.007). The three individual species tested, *H. bilis* (P=0.056), *H. canis* (P=0.018) and *H. marmotae* (P=0.020), were all noted at greater frequency in mucinous adenocarcinoma cases. No significant differences in EHS infection status were noted based on age, sex or breed.

We also examined a group of apparently healthy shelter-origin cats. EHS were prevalent (15/22, 68 %) in these cats, with *H. bilis* (12/22, 55 %), *H. canis* (6/22, 27 %) and *H. marmotae* (6/22, 27 %) all being identified. These cats were free of microscopically apparent intestinal disease.

### DISCUSSION

Prior studies of non-haematopoietic feline intestinal neoplasia have variably described predilections for SI location, male sex, Siamese breed and advanced age (Patnaik et al., 1976; Kosovsky et al., 1988; Turk et al., 1981; Lingeman & Garner, 1972; Cribb, 1988). This report’s large cohort size permitted consideration of patient sex, breed and age as functions of intestinal tumour location and histopathologic classification, further refining prior observations. In this study, LI carcinoma was more frequently observed than SI carcinoma, in contrast with prior reports. Male sex predilection was identified among LI carcinoma cases, while SI carcinoma cases were predominantly female. An association between Siamese breed and intestinal carcinoma could not be confirmed, although Siamese were identified in greater proportion among SI adenocarcinoma cases. Among the three most commonly encountered tumour location categories, LI adenocarcinoma was associated with significantly advanced mean age (mean age, 13 years) when compared to SI adenocarcinoma (mean age, 9 years) and LI mucinous adenocarcinoma (mean age, 9 years). EHS have been identified in numerous animals, including cats, and are associated with experimental intestinal inflammation and neoplasia in rodent models and natural intestinal inflammation and neoplasia in non-human primates. This study examined EHS prevalence in archived intestinal tissue from feline primary intestinal carcinoma cases. One limitation of this approach is that, while the use of archived tissue allowed the assembly of a large number of cases, it did not allow for simultaneous accumulation of matched control tissues. In addition, the use of formalin-fixed tissue forced reliance on FISH-based methodology. While probe validation was performed using both bacterial culture samples and experimentally infected mice, it is possible that probe sensitivity may not have been sufficient to detect low-level EHS infections.

While EHS were identified at high frequency in carcinoma cases, our concurrent analysis of a group of shelter-origin cats suggests that EHS prevalence among cats may be high. This finding is consistent with earlier studies where *H. canis*, *H. bilis* and *H. cinaedi* were identified in healthy and diarrheic cats (Rossi et al., 2008). Others have noted *H. marmotae* in cats used for biomedical research (Fox et al., 2002).

These reports, plus our findings, would mirror the epidemiology of *H. pylori*, which chronically infects half of the human population and causes gastric cancer in a small subset of infected individuals. It is possible that a subset of cats could develop intestinal neoplasia following chronic persistent infection. The high EHS prevalence in cats is consistent with that noted in species such as rhesus macaques and immunodeficient mice where EHS infection and neoplasia have been previously associated (Lertpiriyapong et al., 2014; Fox et al., 2001; Maggio-Price et al., 2006; Erdman et al., 2009). The presence of EHS also suggests that the intestinal microbiota may play an important role in feline intestinal neoplasia.

---

**Table 2. Proportion of cats whose intestinal sections were FISH positive for *Helicobacter* spp., *H. bilis*, *H. canis* or *H. marmotae***

Data shown are pooled and based on intestinal location and histopathologic characteristics. Superscript symbols indicate significantly higher numbers of FISH-positive cases (*P*≤0.05) associated with large intestinal tumour location or mucinous adenocarcinoma morphology. *P*≤0.05 for their comparison.

<table>
<thead>
<tr>
<th></th>
<th><em>Helicobacter</em> spp.*</th>
<th><em>H. bilis</em></th>
<th><em>H. canis</em></th>
<th><em>H. marmotae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal carcinoma, all cases</td>
<td>30/54 (56 %)</td>
<td>22/54 (41 %)</td>
<td>5/54 (9.2 %)</td>
<td>11/54 (20 %)</td>
</tr>
<tr>
<td>Intestinal carcinoma, by intestinal location</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small intestine</td>
<td>6/18 (33 %)†</td>
<td>3/18 (17 %)‡</td>
<td>0/18 (0 %)</td>
<td>3/18 (17 %)</td>
</tr>
<tr>
<td>Large intestine</td>
<td>21/31 (68 %)†</td>
<td>16/31 (52 %)‡</td>
<td>4/31 (13 %)</td>
<td>5/31 (16 %)</td>
</tr>
<tr>
<td>Intestinal carcinoma, by histopathologic type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solid carcinoma</td>
<td>2/5 (40 %)§</td>
<td>1/5 (20 %)#</td>
<td>0/5 (0 %)‖</td>
<td>1/5 (20 %)‖</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>14/28 (50 %)§</td>
<td>11/28 (39 %)#</td>
<td>1/28 (3.5 %)‖</td>
<td>3/28 (11 %)‖</td>
</tr>
<tr>
<td>Mucinous adenocarcinoma</td>
<td>12/13 (92 %)§</td>
<td>9/13 (69 %)#</td>
<td>4/13 (31 %)‖</td>
<td>6/13 (46 %)‖</td>
</tr>
</tbody>
</table>

* Eight cats with intestinal carcinoma and seven cats not diagnosed with intestinal carcinoma were FISH positive by more than one species-specific assay.
EHS were identified significantly more frequently in cats with LI carcinoma. This supports a role for EHS in carcinoma development because the ICC junction and proximal colon are the typical sites for EHS colonization and associated neoplasia in mouse models and naturally infected rhesus macaques (Maggio-Price et al., 2006; Lertpiriyapong et al., 2014; Fox et al., 1999; Kullberg et al., 1998). This study noted increased frequency of LI carcinoma compared to SI carcinoma in contrast to prior reports. It is possible that this topological shift was driven by EHS infection, which is prevalent among these cats. EHS infection was also noted significantly more frequently in cats with mucinous adenocarcinoma. This tumour morphotype has been observed in *H. hepaticus*-infected mice (Maggio-Price et al., 2006; Erdman et al., 2003a, b), as well as rhesus macaques naturally infected with *Helicobacter marmotae* (Lertpiriyapong et al., 2014; Shen et al., 2009).

While this study does not prove a causal relationship between EHS infection and intestinal carcinoma, there are several mechanisms by which this could occur. First, *H. canis*, *H. bilis* and *H. marmotae* produce cytotoxic distending toxin (cdt), which has DNAse activity and causes double-stranded breaks (Chien et al., 2000; Shen et al., 2014). While *H. hepaticus* mutants lacking cdt activity are able to colonize mice, hyperplasia and inflammatory changes are reduced in mouse inflammatory bowel disease models (Young et al., 2004). Second, EHS infection results in phagocyte infiltration at sites of infection, which produces reactive nitrogen, oxygen and halogen species in the intestinal epithelium (Mangerich et al., 2012; Erdman et al., 2009). Specifically, the onset of intestinal cancer in *H. hepaticus*-infected 129 Rag2 knockout mice is driven by neutrophils and causes DNA and RNA damage, primarily through halogenation (Mangerich et al., 2012). Interestingly, the molecular signature of these damage products and serum cytokines, chemokines and acute phase proteins is similar between *H. hepaticus*-infected mice and human IBD patients (Knutson et al., 2013). While no evidence of IBD was identified in the examined sections, prior inflammation or inflammation at other intestinal locations cannot be excluded given the persistent nature of *Helicobacter* spp. infection.

ACKNOWLEDGEMENTS

This work was supported by NIH T32 RR007036, NIH R01 OD011141, NIH P01 CA028842 and NIH P30 ES02109. We would like to thank Alyssa Terestre for her assistance with manuscript preparation.

REFERENCES


