Comparison of endoscopic and full-thickness biopsy specimens for diagnosis of inflammatory bowel disease and alimentary tract lymphoma in cats

Sarah E. Evans, DVM; Jennifer J. Bonczynski, DVM, DACVS; John D. Broussard, DVM, DACVIM; Eveline Han, VMD, DACVIM; Keith E. Baer, DVM, DACVP

Objective—To evaluate the accuracy of endoscopic biopsy (EB) specimens for diagnosis of alimentary tract lymphosarcoma in cats.

Design—Prospective study.

Animals—22 cats with inflammatory bowel disease (IBD) or alimentary tract lymphosarcoma.

Procedures—Endoscopic biopsy specimens were obtained during endoscopy of the stomach and duodenum immediately prior to laparotomy or laparoscopic surgery, during which full-thickness biopsy (FTB) specimens were obtained. Accuracy of histopathologic diagnoses was compared between EB and FTB specimens.

Results—Lymphosarcoma was diagnosed in 10 cats on the basis of FTB specimens. Lymphosarcoma was detected in the jejunum and ileum in all 10 cats, in the duodenum in 9 cats, and in the stomach in 4 cats. In the same 10 cats, EB findings indicated a diagnosis of lymphosarcoma in 3 cats and were suggestive but inconclusive for lymphosarcoma in 3 cats. Lymphosarcoma was correctly diagnosed via gastric EB specimens in 3 of the 4 cats with gastric lymphosarcoma but evaluation of EB specimens led to an incorrect diagnosis of IBD in 4 cats with small intestinal lymphosarcoma.

Conclusions and Clinical Relevance—EB specimens were useful for diagnosis of gastric lymphosarcoma but were not adequate for differentiating between IBD and lymphosarcoma in the small intestine. Because the most common sites of alimentary tract lymphosarcoma in cats are the jejunum and ileum, FTB specimens of those sites should be obtained via laparotomy or laparoscopy for accurate diagnosis. Laparoscopy may be a minimally invasive alternative to endoscopy and laparotomy for obtaining diagnostic biopsy specimens. (*J Am Vet Med Assoc* 2006;229:1447–1450)

Abbreviations

IBD	Inflammatory bowel disease
FTB	Full-thickness biopsy
EB	Endoscopic biopsy
ALT	Alanine transaminase

Inflammatory bowel disease and alimentary tract lymphosarcoma are common conditions that cause chronic gastrointestinal tract disease in cats. Clinical signs include weight loss, vomiting, diarrhea, and variation in appetite. Differential diagnoses include dietary indiscretion; parasitism; hyperthyroidism; and disease of the liver, kidneys, and pancreas. After an extensive series of diagnostic tests and interventions that includes dietary trials, deworming, imaging, and blood tests, IBD and lymphosarcoma are often the remaining differentials. Definitive diagnosis of IBD and lymphosarcoma requires histologic evaluation of gastrointestinal biopsy specimens, which may be obtained via endoscopy, laparotomy, or laparoscopy.[1,2]

Advantages and disadvantages are associated with each diagnostic procedure. Surgical laparotomy enables full inspection and biopsy of the gastrointestinal tract and other abdominal organs. However, laparotomy is invasive, time consuming, expensive, and possibly associated with greater morbidity. Laparoscopy is a less invasive surgical procedure that also allows for exploration of the abdomen and acquisition of FTB specimens, but with less morbidity than laparotomy.[1,2] Endoscopy is the least invasive technique; this procedure allows the endoscopist to view the gastrointestinal tract mucosa and necessitates a shorter period of hospitalization.[3] Disadvantages of endoscopy include inability to obtain FTB specimens, evaluate and biopsy other abdominal organs, or gain access to the jejunum and ileum in most cats. Proper instrumentation and biopsy technique are essential for acquisition of adequate tissue samples via EB.[4-6]

Although histologic evaluation is necessary to establish a diagnosis of IBD or lymphosarcoma, problems in making the diagnosis may be encountered because of overlap in histologic features between the 2 entities, differences of opinion among pathologists, and inadequacy of tissue specimens.[2,7-9] The potential for progression of IBD to lymphosarcoma further complicates diagnosis.[10] Among the key challenges associated with gastrointestinal tract biopsy is the need to obtain tissue at the correct location and of adequate depth. It is recommended that 4 samples from each site be submitted when EB is performed, whereas a single FTB specimen from each site is sufficient.[4-6] Histologically, IBD is characterized by diffuse inflammatory cell infiltration of the mucosal layer. These cell populations are typically dominated by lymphocytes and plasma cells but may also include eosinophils, neutrophils, and macrophages. Criteria for diagnosis of lymphoma have been described and include mucosal infiltration by neoplastic lymphocytes with frequent progression to submucosal and transmural infiltra-

From the E & M Bobst Hospital and Caspary Research Institute of The Animal Medical Center, 510 E 62nd St, New York, NY 10021. Dr. Evans' present address is Hollywood Animal Hospital, 2864 Hollywood Blvd, Hollywood, FL 33020.

Supported in part by a grant from the WINN Feline Foundation.

Presented in part at the 32nd American College of Veterinary Surgeons Veterinary Symposium, Washington, DC, October 2006.

Address correspondence to Dr. Evans.

tion.[8,11] Endoscopic biopsy, in which the depth of tissue removal is limited to the mucosal layer, may not permit detection of lymphosarcoma in deeper tissues. Clinically, the most common site for lymphosarcoma appears to be the ileocecocolic junction, which may be difficult or impossible to reach endoscopically.[1,8] Therefore, diagnosis of lymphosarcoma may require examination of FTB specimens obtained via laparotomy or laparoscopy. To the authors' knowledge, no previous studies supporting the diagnostic accuracy of mucosal EB specimens for distinguishing between IBD and lymphosarcoma have been published. At present, histologic evaluation of FTB specimens is considered the gold standard for diagnosis of lymphosarcoma. In this study, we compared diagnoses made on the basis of EB specimens with those made on the basis of FTB specimens to assess the accuracy of EB in diagnosing lymphosarcoma in cats.

Materials and Methods

Cats—Cats examined at The Animal Medical Center from November 2004 to December 2005 with clinical signs of chronic gastrointestinal tract disease (eg, vomiting, small intestinal diarrhea, change in appetite, or weight loss) were eligible for the study if results of a minimum diagnostic database (ie, CBC; serum biochemical analyses; assessment of serum T4 concentration, serum trypsinlike immunoreactivity, serum folate and cobalamin concentrations; fecal examination; radiography; and ultrasonographic examination) indicated IBD or neoplasia (alimentary tract lymphosarcoma) as the most likely diagnoses.

Cats with discrete abdominal masses and those for which the minimum database was not completed or in which endoscopy was not successfully performed were excluded from the study. Lymphadenopathy was not considered a criterion for exclusion. Signed, informed client consent was obtained for all cats enrolled in the study.

Biopsy procedures—Cats were premedicated with glycopyrrolate (0.01 mg/kg [0.005 mg/lb], IM) or atropine (0.02 mg/kg [0.01 mg/lb], IM); anesthesia was induced with propofol (3 to 6 mg/kg [1.36 to 2.73 mg/lb], IV) and diazepam (0.5 mg/kg [0.23 mg/lb], IV) administered to effect and maintained with inhaled isoflurane or sevoflurane and oxygen. Gastroduodenoscopy to obtain gastrointestinal biopsy specimens was performed by an endoscopist (JJB or EH). A video gastroscope[a] with a 100-cm insertion tube, 9.5-mm outer diameter, 2.8-mm working channel, and 2.6-mm flexible biopsy forcep was used. Mucosal surfaces of the esophagus, stomach, and duodenum were evaluated, and gross lesions were recorded. Representative specimens of the stomach (n = 8) and duodenum (6) were obtained for histologic evaluation in each cat. When duodenal intubation was not possible, at least 3 duodenal specimens were obtained blindly.

Morphine-derived analgesics (eg, butorphanol [0.2 mg/kg {0.09 mg/lb}, IV], buprenorphine [0.03 mg/kg {0.014 mg/lb}, IV], or hydromorphone [0.05 mg/kg {0.023 mg/lb}, IV]) were administered at the conclusion of endoscopy and prior to surgery. Immediately following endoscopy, exploratory laparotomy or laparoscopy was performed and FTB specimens of the stomach, duodenum, jejunum, and ileum were obtained. Surgical procedures were performed by staff surgeons or surgery residents supervised by staff surgeons. Liver, pancreas, and enteric lymph nodes were biopsied in addition to other organs that had a grossly abnormal appearance. Observations of gross abnormalities of the abdominal organs were recorded. The biopsy sites and abdominal incision were closed routinely. Postoperative care was routine for cats undergoing gastrointestinal tract surgery.

Biopsy specimens were labeled and placed in jars containing 10% formalin solution and submitted for histologic examination. Histopathology slides were evaluated by a single pathologist (KEB). All FTB specimens were submitted immediately after surgery and were evaluated by the pathologist before the EB specimens were evaluated. To prevent bias in evaluation of the EB specimens, specimens were submitted in batches at least 2 weeks after the most recent FTB submission. The pathologist was unaware of endoscopic and surgical observations and results of FTB specimen analysis while examining the EB specimens.

All specimens were evaluated for inflammatory, neoplastic, and other lesions. Histologic evaluations were similar for full-thickness and endoscopic gastric and intestinal specimens. Inflammatory bowel disease was diagnosed when varying degrees of mucosal and submucosal infiltration by inflammatory cells were observed. Mixed populations of lymphocytes and plasma cells were typically present, and small lymphocytes were the most frequent dominant cell type. Lymphosarcoma was diagnosed when there was mucosal infiltration and expansion in EB specimens and when there was frequent infiltration into deeper layers (ie, into the submucosa and muscle wall) by neoplastic lymphocytes in FTB specimens. The neoplastic lymphocyte population was essentially monotonous, with few plasma cells and occasional eosinophils. Specimens were considered to be suggestive of lymphosarcoma when marked mucosal infiltration by a monotonous population of well-differentiated lymphocytes and very low numbers of other inflammatory cells were detected.

Statistical analysis—The Fisher exact χ^2 test was used to assess the sensitivity of the diagnosis of lymphosarcoma and IBD for EB specimens, compared with FTB specimens. Statistical calculations were performed with commercially available software.[b] Values of $P < 0.05$ were considered significant.

Results

Twenty-two cats were included in the study. Of those, twelve cats had IBD and 10 cats had alimentary tract lymphosarcoma on the basis of histopathologic findings on FTB specimens. Although the difference was not significant, cats with IBD (mean age, 9.7 years; range, 1.5 to 16 years) were slightly younger than cats with lymphosarcoma (mean age, 12.5 years; range, 10 to 15 years). There was no sex or breed predilection in either group.

Owner complaints at examination were not different between the 2 groups. The most common owner complaint for cats in both groups was weight loss. All cats with lymphosarcoma had lost weight over the course of 1 year or longer. In addition to weight loss, 7 of the 10 cats with lymphosarcoma had vomiting, diarrhea, or both at initial examination and 1 of the 10 had decreased appetite. Eight of the 12 cats with IBD had weight loss of less than 10 months' duration at initial examination. Among the 12 cats with IBD, 3 had inappetence and 9 had vomiting, diarrhea, or both at initial examination (**Table 1**).

No differences in physical examination findings were detected between the 2 groups. On initial examination in cats with lymphosarcoma, the intestines were palpably thick in 5 cats and abdominal lymph nodes were enlarged in 2 cats. Five of the 10 cats had a body condition score of 4 of 9 or higher, and 4 cats had a body condition score ≤ 3 of 9. In cats with IBD, the intestines were palpably thick in 3 cats and the abdom-

Table 1—Mean age and numbers of cats with various preoperative findings in 22 cats with IBD or alimentary tract lymphosarcoma that underwent EB and surgical FTB of gastrointestinal tract segments.

Variable	IBD (n = 10)	Lymphosarcoma (12)
Age (y)	9.7	12.5
Vomiting	4	3
Diarrhea	3	3
Weight loss	6	10
Inappetence	3	1
BCS ≤ 3/9	5	4
BCS ≥ 4/9	5	5
Thick GI loops on PE	3	5
Large LN on PE	1	2
Low serum cobalamin concentration	3	6
Low serum folate concentration	4	0
High serum ALT activity	1	5
Thick GI loops on ultrasonography	9	8
Large LN on ultrasonography	2	5

BCS = Body condition score. GI = Gastrointestinal. PE = Physical examination. LN = Lymph node.

inal lymph nodes were enlarged in 1 cat. Five of the 12 cats had a body condition score ≥ 4 of 9, and 5 cats had a body condition score ≤ 3 of 9. A body condition score was not recorded for 2 cats with IBD (Table 1).

Of the initial diagnostic laboratory tests, high serum ALT activity was the only factor for which the difference between cats with IBD and those with lymphosarcoma approached significance (P = 0.055). Serum ALT activity was high in 5 of the 10 cats with lymphosarcoma and in 1 of the 12 cats with IBD. All 6 cats with high serum ALT activity also had histopathologic changes in the liver.

Abdominal ultrasonographic findings in the 10 cats with lymphosarcoma included thick muscularis and submucosal layers in the small intestine (n = 7 cats), lymphadenopathy (6), changes consistent with pyelonephritis (2), thick small intestinal mucosa (1), and thick stomach wall (1). Five of the 6 cats with lymphosarcoma that had mesenteric lymphadenopathy as detected ultrasonographically were also determined to have lymphosarcoma in the lymph nodes. The stomach appeared thick in only 1 of the 4 cats with gastric lymphosarcoma. Ultrasonographic findings in the 12 cats with IBD included thick muscularis and submucosal layers in the small intestine (n = 4 cats), thick small intestinal mucosa (3), lymphadenopathy (2), thick stomach wall (2), and changes consistent with cholecystitis (1). The ultrasonographic appearance of the liver and pancreas was considered normal in all cats.

Endoscopic biopsy specimens were judged to be adequate for evaluation by the pathologist in all gastric samples and in 20 of 22 duodenal samples. In 4 cats with a diagnosis of gastric lymphosarcoma on the basis of FTB findings, EB specimens were diagnostic for lymphosarcoma in 3 cats and suggestive of lymphosarcoma in 1 cat. In 2 other cats, findings were suggestive of gastric lymphosarcoma on an EB specimen but IBD was diagnosed on the basis of FTB specimens. One of those cats had small intestinal lymphosarcoma. Of 9 cats with a diagnosis of duodenal lymphosarcoma on the basis of FTB findings, 1 had a diagnosis of lymphosarcoma on EB, 3 had findings suggestive of lymphosarcoma, and 5 had a diagnosis of IBD on EB. Of the 5 cats with lymphosarcoma diagnosed as IBD on duodenal EB, 2 cats had only partial duodenal assessment and in 3 cats, duodenal biopsy had been performed blindly. Of the 10 cats with lymphosarcoma in any area (ie, stomach, intestines, lymph node, liver, or pancreas), lymphosarcoma was also diagnosed on EB specimens in 3 cats; findings were suggestive of but inconclusive for lymphosarcoma in 3 other cats, and IBD was incorrectly diagnosed in 4 cats.

Full-thickness biopsy with laparoscopic assistance was performed in 4 of the cats with lymphosarcoma and 3 of the cats with IBD. Laparotomy was performed to obtain FTB specimens in 6 cats with lymphosarcoma and 9 cats with IBD. At surgery, 8 of the 10 cats with lymphosarcoma had thick portions within the gastrointestinal tract; lymph nodes were large in 8 of those 10 cats, and the liver had a mottled appearance in 2 cats. Surgical findings in the 12 cats with IBD included thick areas of the gastrointestinal tract in 6 cats, mesenteric lymphadenopathy in 7 cats, mottled liver in 3 cats, and pancreatic nodules in 1 cat.

Lymphosarcoma was detected in both the jejunum and ileum of all 10 cats that had a diagnosis of lymphosarcoma. Lymphosarcoma also involved the duodenum in 9 of those 10 cats and was detected in the stomach of 4 of the cats. Lymphosarcoma was detected in lymph nodes, liver, or both in all of the cats with a diagnosis of lymphosarcoma.

In cats with IBD, additional histopathologic findings included cholangiohepatitis (n = 3 cats), lymphoid hyperplasia (3), portal hepatitis (2), portal fibrosis (2), and pancreatitis (2). Of the 2 cats with pancreatitis, disease was acute in 1 cat and chronic in the other. Portal hepatitis and portal fibrosis were also detected in the cat with chronic pancreatitis.

Results of a Fisher exact χ^2 test confirmed a significant (P = 0.003) difference between specimens derived by FTB and those obtained via EB in yielding a diagnosis of lymphosarcoma. There was no significant difference in diagnosis of gastric lymphosarcoma between EB and FTB specimens (P = 0.285). However, results of χ^2 analysis indicated that there was a significant ($P < 0.001$) difference between EB and FTB duodenal specimens.

Discussion

Cats in this study comprised a unique population that was selected for clinical conditions compatible with a diagnosis of IBD. However, nearly half were found to have lymphosarcoma. This finding calls into question the common practice of presumptively diagnosing IBD on the basis of clinical signs and results of noninvasive diagnostic tests or therapeutic trials. It also highlights our inability to select cases that are appropriate for less invasive (ie, endoscopic) diagnostic procedures. No correlation was found between diagnosis of lymphosarcoma and clinical signs, physical examination findings, abdominal ultrasonographic findings, or endoscopic or surgical observations; thus, there may be no clinical basis for differentiating between lymphosarcoma and IBD.

Ultrasonographic imaging is commonly used in evaluation of cats with chronic gastrointestinal disease and is important in screening for neoplasia manifested by structural disease (masses). In our study population of cats that did not have mass-forming disease, ultrasonography was not helpful in differentiating between lymphosarcoma and IBD. Ultrasonography was also not helpful in establishing the presence or absence of disease involving the lymph nodes, pancreas, or liver.

No surgical complications occurred after any of the laparotomy or laparoscopic procedures, a finding that is likely attributable to strict adherence to proper surgical technique to avoid intraoperative contamination, postoperative gastrointestinal tract leakage, and dehiscence. Laparoscopy was an effective minimally invasive alternative to laparotomy for abdominal exploration and was useful for assisting in biopsy of abdominal organs, including FTB of the stomach and intestine.

Veterinarians and clients often choose to obtain EB specimens of the stomach and duodenum rather than FTB specimens so that surgery can be avoided. However, results indicated that EB specimens yielded an incorrect or inconclusive diagnosis in as many as 9 of 11 cats. Misdiagnosis results in inappropriate and ineffective treatment and permits progression of the disease. Prompt diagnosis and proper treatment are imperative for the best possible outcome, particularly with neoplastic diseases.

Mean procedure time for EB was < 20 minutes, and no complications were reported. Grossly evident mucosal changes were not predictive of lymphosarcoma or IBD. Duodenal assessment was limited in half the cats, and biopsy was performed blindly in 8 of the cats. Limited assessment of duodenal tissues likely contributed to the poor sensitivity of EB for detecting duodenal lymphosarcoma. This may have been in part a result of use of an endoscope with a large insertion tube (9.5-mm diameter). However, the large (2.8-mm diameter) working channel of this endoscope may have permitted collection of better biopsy specimens. All gastric biopsy specimens and 21 of the duodenal biopsy specimens were judged by the pathologist to be adequate for evaluation.

Results indicated that EB is a sensitive technique for diagnosis of gastric lymphosarcoma in cats. However, although EB led to the correct diagnosis of gastric lymphosarcoma in 3 of 4 cats, less than half of the cats with lymphosarcoma had gastric lymphosarcoma. Correlations were not found between any preoperative data and diagnosis of gastric lymphosarcoma versus small intestinal lymphosarcoma or IBD; before histopathologic results were known, a diagnosis of gastric lymphosarcoma was no more likely than a diagnosis of small intestinal lymphosarcoma or IBD. Although EB specimens were diagnostic for lymphosarcoma in a third of the cats with lymphosarcoma, findings were suggestive of but inconclusive for lymphosarcoma in an additional third of the cats that had lymphosarcoma (duodenal in 2 cats and gastric in 1 cat). Therefore, EB may not be an accurate means for diagnosing small intestinal lymphosarcoma in cats.

Endoscopic biopsy specimens of the stomach and duodenum were not adequate for differentiating between IBD and lymphosarcoma in the present study. Obtaining EB specimens of the jejunum and ileum may improve the diagnostic sensitivity of EB and may therefore be an alternative to obtaining FTB specimens for diagnosis of lymphosarcoma in cats. However, the same difficulties in differentiating between small cell lymphosarcoma and IBD still apply with such specimens. Moreover, EB of the jejunum and ileum can be difficult or impossible because of limitations of the equipment. Laparoscopy is a minimally invasive surgery that may be a useful means of obtaining FTB specimens required for accurate diagnosis.

a. Olympus America, GIF 100, Mellville, NY.
b. QuikCalcs and Prizm 4.0, GraphPad Software Inc, San Diego, Calif.

References

1. Tams TR. Chronic feline inflammatory bowel disorders. Part II. Feline eosinophilic enteritis and lymphosarcoma. *Compend Contin Educ Pract Vet* 1986;8:464–470.
2. Wasmer ML, Willard MD, Helman RG, et al. Food intolerance mimicking alimentary lymphosarcoma. *J Am Anim Hosp Assoc* 1995;31:463–466.
3. Mansell J, Willard MD. Biopsy of the gastrointestinal tract. *Vet Clin North Am Small Anim Pract* 2003;33:1099–1116.
4. Willard MD, Lovering SL, Cohen ND, et al. Quality of tissue specimens obtained endoscopically from the duodenum of dogs and cats. *J Am Vet Med Assoc* 2001;219:474–479.
5. Zoran DL. Gastroduodenoscopy in the dog and cat. *Vet Clin North Am Small Anim Pract* 2001;31:631–656.
6. Twedt DC. Perspectives in gastrointestinal endoscopy. *Vet Clin North Am Small Anim Pract* 1993;23:481–495.
7. Roth L, Leib MS, Davenport DJ, et al. Comparisons between endoscopic and histologic evaluation of the gastrointestinal tract in dogs and cats: 75 cases (1984–1987). *J Am Vet Med Assoc* 1990;196:635–638.
8. Richter KP. Feline gastrointestinal lymphoma. *Vet Clin North Am Small Anim Pract* 2003;33:1083–1098.
9. Willard MD, Jergens AE, Duncan RB, et al. Interobserver variation among histopathologic evaluations of intestinal tissues from dogs and cats. *J Am Vet Med Assoc* 2002;220:1177–1182.
10. Davenport DJ, Leib MS, Roth L. Progression of lymphocytic-plasmacytic enteritis to gastrointestinal lymphosarcoma in three cats, in *Proceedings*. Vet Cancer Soc 1987;suppl.
11. Gabor LJ, Canfield PJ, Malik R. Immunophenotypic and histological characterization of 109 cases of feline lymphosarcoma. *Aust Vet J* 1999;77:436–441.