STANDARD ARTICLE

Journal of Veterinary Internal Medicine

ACVIM
American College of
Veterinary Internal Medicine

Ability of ultrasonography to predict the presence and location of histologic lesions in the small intestine of cats

Talia Guttin[1] | Audra Walsh[2] | Amy C. Durham[3] | Jennifer A. Reetz[4] |
Dorothy C. Brown[5] | Mark P. Rondeau[4]

[1]Department of Small Animal Medicine and Surgery, St. George's University School of Veterinary Medicine, Grenada, West Indies

[2]VetPath Laboratories, Perth, Western Australia, Australia

[3]Department of Pathobiology, University of Pennsylvania School of Veterinary Medicine, Philadelphia, Pennsylvania

[4]Department of Clinical Sciences and Advanced Medicine, University of Pennsylvania School of Veterinary Medicine, Philadelphia, Pennsylvania

[5]Martingale Consulting, Media, Pennsylvania

Correspondence
Talia Guttin, Department of Small Animal Medicine and Surgery, St. George's University School of Veterinary Medicine, True Blue, Grenada, West Indies.
Email: tguttin@sgu.edu

Abstract

Background: Diagnosis of infiltrative small intestinal (SI) disease in cats is challenging, and debate continues regarding optimal biopsy techniques. Ultrasonography may facilitate selection of biopsy type and location.

Hypothesis/Objectives: Assess ability of ultrasonography to predict histologic lesions by SI segment and tissue layer.

Animals: One-hundred sixty-nine cats that had abdominal ultrasonography and full-thickness SI biopsies performed.

Methods: Ultrasonographic images and full-thickness biopsy samples were retrospectively reviewed, and each SI wall layer evaluated for lesions according to published standards.

Results: Ultrasonographic SI lesions were present in 132 cats (63 duodenum; 115 jejunum; 71 ileum). Samples were obtained at laparotomy (60) or necropsy (109). Ultrasonographic abnormalities had high positive predictive value (PPV) for histologic lesions (duodenum, 82.0%; 95% confidence interval [CI], 68.6-91.4; jejunum, 91.0%; 95% CI, 81.5-96.6; ileum, 88.1%; 95% CI, 74.4-96.0), but poor negative predictive value (duodenum, 27.1%; 95% CI, 17.2-39.1; jejunum, 27.3%; 95% CI, 10.7-50.2; ileum, 40.4%; 95% CI, 26.4-55.7). The ability of ultrasonography to predict histologic lesions in this population, which had high disease prevalence (SI histologic lesions in 78.1% of cats) was high for mucosal lesions (PPV, 72.7%-100%) but low for submucosal or muscularis lesions (PPV, 18.9%-57.1%).

Conclusions and Clinical Importance: In a population with high disease prevalence, most cats with SI mucosal ultrasonographic lesions will have mucosal histologic lesions. Small intestinal submucosal and muscularis ultrasonographic lesions are not predictive of histologic disease in those layers, suggesting that full-thickness biopsy may not be essential in these cats. Ultrasonography may help guide decisions about biopsy type in individual cats.

KEYWORDS
biopsy, endoscopy, enteropathy, histopathology

1 | INTRODUCTION

Infiltrative small intestinal (SI) disease in cats poses a diagnostic challenge for clinicians. Specifically, differentiating between inflammatory bowel disease (IBD) and small-cell lymphosarcoma/alimentary lymphoma (SC-LSA) can be difficult. These 2 SI diseases can be identical in history, presentation, physical examination findings, clinicopathologic changes, and ultrasonographic findings.[1–7] Small intestinal histopathology is the gold standard for diagnosing and differentiating these 2 disease entities. Because treatment and prognosis vary, differentiating between these 2 diseases is crucial.[3,8] However, the diagnostic gold standard still carries with it 2 inherent problems: how best to obtain biopsy specimens (endoscopic versus full thickness) and consistency in histologic interpretation of the biopsy specimens.[6,9]

A debate has emerged within the field of veterinary gastroenterology in recent years over how to obtain diagnostic biopsy specimens from the feline SI, by endoscopy or full thickness surgical biopsy. Many recent studies have brought the diagnostic utility of endoscopic biopsy into question and shown high variation among histologic diagnoses in different segments of SI.[2,5,7,10–13] The previous studies, however, have failed to consider the biopsy collection methodology used for each individual case. It has been stated that ultrasonography of the abdomen should be performed before endoscopy to make sure the lesion of interest is not out of reach, such as mid-jejunum, but the utility of ultrasonography to predict the presence and location of histologic lesions has not been thoroughly evaluated.[14]

The diagnostic utility of ultrasonography has been assessed in studies comparing ultrasonographic lesions to a specific diagnosis in dogs.[15,16] Other studies have found that ultrasonography of the SI in cats accurately assesses the 4 layers of the SI, and normal values for overall thickness and individual layer thickness have been reported.[4,17–19] Some specific ultrasonographic lesions of the SI correlate with specific histologic changes. For example, smooth muscle thickening on ultrasonography correlates with smooth muscle hypertrophy, and striations of the mucosa on ultrasonography correlate with histologic lacteal dilatation.[20,21] Ultrasonography also can assess functional versus mechanical ileus, and the hemodynamics of the gastrointestinal tract.[22] However, specific ultrasonographic lesions differentiating among different forms of infiltrative SI diseases in cats have proven more controversial. The ultrasonographic findings of SI muscularis thickening and lymphadenopathy in cats were correlated with SC-LSA in 1 study.[4] However, SI muscularis thickening has been reported in cats with eosinophilic enteritis, IBD, and SC-LSA and in cats without clinical evidence of gastrointestinal disease in other studies.[11,19,23]

The purpose of our study was to assess the ability of ultrasound examination to predict the presence and location of histologic lesions by segment and tissue layer of the SI. Ultimately, if ultrasonography does have diagnostic utility in the localization of disease, the clinician can use this information to choose the best biopsy method for each individual patient and increase the diagnostic yield of the biopsy specimens.

2 | MATERIALS AND METHODS

Veterinary records of the Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania from 2007 to 2013 were searched to select cats that had an abdominal ultrasonographic examination performed and full-thickness SI biopsy, necropsy, or both performed within 33 days after the initial ultrasonographic examination. Data collected included signalment, weight, clinical signs, and physical examination findings. For clinical signs, gastrointestinal signs were defined as 1 of any of the following: vomiting, diarrhea, anorexia, or weight loss. Clinical signs were classified as chronic if their duration was longer than 3 weeks.[14] If clinical signs were present for ≤3 weeks, they were classified as acute signs.

A single board-certified radiologist (J.A. Reetz) blinded to the final diagnosis reviewed all the available ultrasonographic images of the SI tract, which included primarily still images with occasional short cine loops. By utilizing measurement calipers on digital imaging and communications in medicine viewing software (Philips iSite Radiology 3.5.0; Royal Philips Electronics, Amsterdam, The Netherlands), measurements of total intestinal wall thickness (mucosal interface through serosa) and each individual layer were performed (mucosa, submucosa, muscularis, serosa) of the duodenum, jejunum, and ileum, when images were available. Measurements were performed on longitudinal images except for the ileum, where measurements were performed on transverse images whenever possible as described in a prior study.[18] For the jejunum, a muscularis:submucosa ratio also was recorded. If intestinal wall layering was absent, only the total wall thickness was recorded. From these measurements, total wall and individual layer thickness and jejunal muscularis:submucosa ratio were listed as normal or abnormal, using previous references as a guide.[4,17–19] Thickness was considered increased if total wall thickness was >2.5 mm for the duodenum and jejunum, and >3.2 mm for the ileum; mucosa was >1.5 mm for the duodenum and jejunum, and >0.6 mm for the ileum; submucosa was >0.4 mm for the duodenum and jejunum, and >2 mm for the ileum; muscularis was >0.3 mm for the duodenum, >0.4 mm for the jejunum, and >0.9 mm for the ileum; and jejunal muscularis:submucosa ratio was >1. Finally, intestinal mural alteration aside from thickening, such as visualization of a hyperechoic mucosal band, was recorded. See Figure 1 for a demonstration of wall layer measurement and common ultrasonographic lesions. If no images of a particular segment were available, the ultrasound examination reports were reviewed for comments regarding the SI (ie, if they were described as normal or abnormal), but because no measurements could be retrospectively obtained, these segments were not included in statistical analysis.

All histologic samples were assessed by a single anatomic pathology resident (A. Walsh) with direct supervision by a board-certified anatomic pathologist (A.C. Durham), both blinded to the ultrasound examination findings. Histologic samples were graded and documented based exclusively on WSAVA International Gastrointestinal Standardization Group histopathology standards.[9] For each of the aforementioned intestinal samples evaluated, anatomical regions (eg, mucosa,

1280 | Journal of Veterinary Internal Medicine

ACVIM
Open Access
American College of
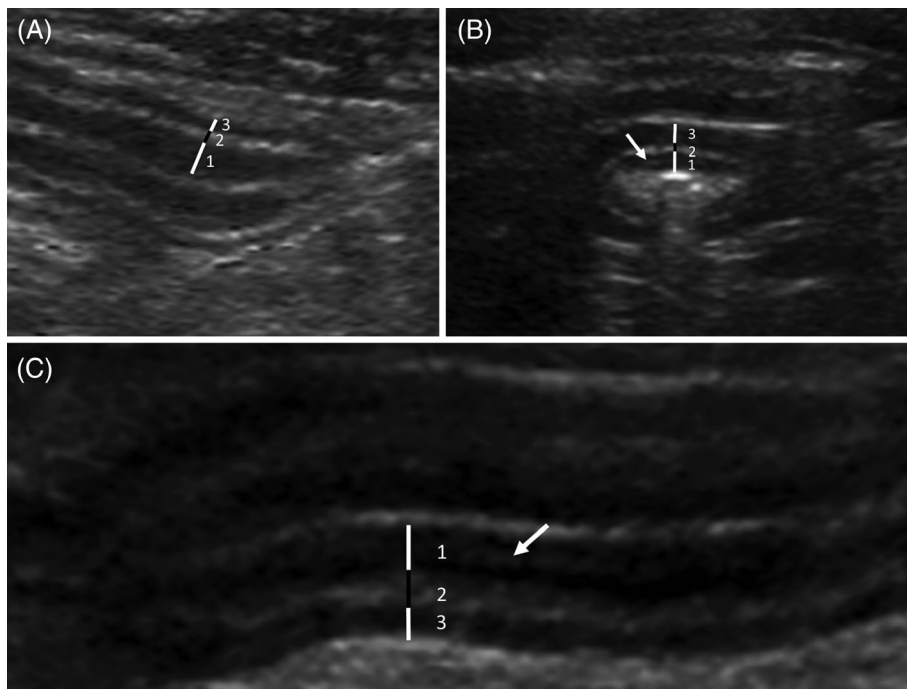Veterinary Internal Medicine

GUTTIN ET AL.

FIGURE 1 Ultrasonographic images of the jejunum of 3 different cats, demonstrating measurement of individual wall layers (1 = mucosa, 2 = submucosa, 3 = muscularis). A, Lymphocytic-plasmacytic enteritis on necropsy but normal wall thickness and layering differentiation. B, Mucosal fibrosis and smooth muscle hypertrophy with thickening of the muscularis layer (1 mm); mucosal (0.7 mm) and submucosal (0.4 mm) layer thickness is normal but a hyperechoic mucosal band is present (white arrow). C, Small-cell lymphoma on intestinal biopsy with thickening of the submucosa (1 mm) and muscularis (0.7 mm) layers; mucosal thickness (1.4 mm) is normal but a hyperechoic mucosal band is present (white arrow)

submucosa, muscularis, and serosa) were evaluated and categorized based on the extent of villous stunting, villous epithelial injury, crypt distention, lacteal dilatation, mucosal fibrosis, intraepithelial inflammation, and mucosal inflammation (eg, lymphocytic, plasmacytic, histiocytic, eosinophilic, and neutrophilic). Each of the categories was scored as normal, mild, moderate, or marked, based on the WSAVA histopathology standards.[9] Additionally, 3 measurements of thickness were taken using microscopic camera software (Olympus cellSens) and averaged for each sample (eg, full wall thickness, mucosa, submucosa, muscularis, and serosa), where applicable. Muscularis:submucosa ratio was calculated for each sample obtained, where applicable.

For statistical analysis, an ultrasonographic lesion was defined as the presence of ≥1 of the following: increased thickness, abnormal muscularis:submucosa ratio, altered echogenicity (hyperechoic or hypoechoic), loss of layers, hyperechoic mucosal bands, masses, foreign bodies, or intussusceptions. A histologic lesion was defined as the presence of ≥1 of the lesions defined by the WSAVA histopathology standards, as discussed above. Descriptive statistics were calculated. Continuous variables were described using means and SDs, unless not normally distributed, in which case median values and ranges are reported. Categorical variables were presented as frequencies. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), were calculated using standard equations.

3 | RESULTS

A total of 169 cats met the inclusion criteria. The median time from ultrasound examination to biopsy or necropsy was 1 day (range, 0-33 days). The median age of cats was 9 years (range, 0.5-21 years). There were 92 males and 77 females in the study. Of the 92 males, 6 were intact, and of the 77 females, 5 were intact. There were

20 purebred cats and 149 domestic short, medium, or long-haired cats. Mean body weight was 4.5 ± 1.52 kg. Seventy-two cats (42.7%) were considered underweight, 39 cats (23.3%) were normal weight, and 58 cats (34%) were overweight based on reported body condition scores. Clinical signs included anorexia in 102 of 168 cats (60.7%), vomiting in 83 of 167 cats (50%), weight loss in 58 of 166 cats (35%), and diarrhea in 29 of 167 cats (17.4%). Overall, 121 of 167 cats (72.5%) had at least 1 clinical sign potentially attributable to gastrointestinal disease. Clinical signs were chronic (>3 weeks' duration) in 77 of 164 (47%) cats. Median duration of clinical signs was 14 days (range, 1 to >365). Physical examination findings included abdominal pain in 40 of 162 cats (24.7%), palpable abdominal mass in 23 of 162 cats (14.2%), and palpably thickened SI in 22 of 162 cats (13.6%).

Ultrasonographic SI lesions were present in 132 cats in the duodenum (63), jejunum (115), or ileum (71, Table 1). Biopsy specimens were obtained via laparotomy (60) or necropsy (109). The type and number of ultrasonographic lesions are summarized in Table 1. Diagnoses in

TABLE 1 Types of ultrasonographic lesions by small intestinal segment

	Duodenum	Jejunum	Ileum
Muscularis thickness	42	96	61
Submucosa thickness	14	24	9
Mucosa thickness	11	15	19
Loss of layers	13	22	8
Mucosa hyperechoic	9	23	8
Mucosal bands	3	23	1
Mass	3	10	2
Foreign body	1	7	1
Intussusception	0	1	2

TABLE 2   Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of ultrasonographic lesions in various segments, layers, or both of the small intestinal wall to predict the presence of a histopathologic lesion in the same segment, layer, or both

SI segment and wall layer	Prevalence (95% CI)	Sensitivity (95% CI)	Specificity (95%CI)	PPV (95% CI)	NPV (95% CI)
Duodenum					
Any layer (n = 120)	76.7% (68.1-83.9)	44.6% (34.2-55.3)	67.9% (47.6-84.1)	82.0% (68.6-91.4)	27.1% (17.2-39.1)
Mucosa (n = 114)	72.8% (63.7-80.7)	19.3% (11.4-29.4)	80.6% (62.5-92.5)	72.7% (49.8-89.3)	27.2% (18.4-37.4)
Submucosa (n = 115)	16.5% (10.3-24.6)	36.8% (16.3-61.6)	88.5% (80.4-94.1)	38.9% (17.3-64.3)	87.6% (79.4-93.4)
Muscularis (n = 116)	11.3% (6.2-18.6)	53.8% (25.1-80.8)	73.5% (63.9-81.8)	20.6% (8.7-37.9)	92.6% (84.6-97.2)
Jejunum					
Any layer (n = 89)	86.5% (77.6-92.8)	79.2% (68.5-87.6)	50.0% (21.1-78.9)	91.0% (81.5-96.6)	27.3% (10.7-50.2)
Mucosa (n = 87)	87.5% (78.5-93.5)	40.8% (29.6-52.7)	90.9% (58.7-99.8)	96.9% (83.8-99.9)	18.2% (9.09-30.9)
Submucosa (n = 88)	37.5% (27.4-48.5)	24.2% (11.1-42.3)	89.1% (77.8-95.9)	57.1% (28.9-82.3)	66.2% (54.3-76.8)
Muscularis (n = 88)	33.0% (23.3-43.8)	86.2% (68.3-96.1)	44.1% (31.2-57.6)	43.1% (30.2-56.8)	86.7% (69.3-96.2)
Ileum					
Any layer (n = 89)	73.0% (62.6-81.9)	56.9% (44.0-69.2)	79.2% (57.8-92.9)	88.1% (74.4-96.0)	40.4% (26.4-55.7)
Mucosa (n = 86)	70.9% (60.1-80.2)	21.3% (11.9-33.7)	100% (86.3-100)	100% (75.3-100)	34.2% (23.5-46.3)
Submucosa (n = 86)	27.9% (18.8-38.6)	12.5% (2.7-32.4)	90.3% (80.1-96.4)	33.3% (7.5-70.1)	72.7% (61.4-82.3)
Muscularis (n = 87)	14.9% (8.2-24.2)	53.8% (25.1-80.8)	59.5% (47.4-70.7)	18.9% (8.0-35.2)	88.0% (75.7-95.5)

Prevalence of histopathologic lesions in each segment or layer is provided.
Abbreviations: 95% CI, 95% confidence interval; n, number of cases having both ultrasound and biopsy available for evaluation from each segment or layer.

cats with SI pathology included inflammation consistent with IBD (105), SC-LSA (16), intermediate large-cell lymphoma (14), other neoplasia (13), and suspected feline infectious peritonitis (10). Biopsy identified normal SI in 25 cats. Histologic disease outside the gastrointestinal tract was found in 70% of cats.

Overall, ultrasonographic lesions in the SI in any wall layer had high PPV for histologic SI lesions in any wall layer within the same SI segment (duodenum, 82.0%; 95% confidence interval [CI], 68.6-91.4; jejunum, 91.0%; 95% CI, 81.5-96.6; ileum, 88.1%; 95% CI, 74.4-96.0) but poor NPV (duodenum, 27.1%; 95% CI, 17.2-39.1; jejunum, 27.3%; 95% CI, 10.7-50.2; ileum, 40.4%; 95% CI, 26.4-55.7). Table 2 shows the ability of ultrasonography to predict histologic lesions in specific SI wall layers.

Eighty-six cats had ultrasonographic jejunal lesions with duodenal histopathology available. Of these cats, 83.7% had histologic duodenal lesions, whereas 16.3% had normal duodenal histology. Fifty-five cats with ultrasonographic jejunal lesions had ileal histopathology available. Of these cats, 76.4% had histologic ileal lesions, whereas 23.7% had normal ileal histology. Thirty-five cats only had ultrasonographic SI lesions in the jejunum. Histologically, 22 of the 35 cats (62.9%) had lesions in the duodenum, ileum, or both. The sensitivity, specificity, and predictive values of jejunal ultrasonographic lesions (any wall

layer) to predict histologic lesions in the duodenum or ileum (any wall layer) are shown in Table 3.

Cats with ultrasonographic muscularis lesions were examined for histologic lesions of the mucosa and submucosa within the same SI segment. Results are summarized in Table 4. In all segments, an ultrasonographic muscularis lesion had high PPV for a mucosal histologic lesion.

4 | DISCUSSION

We assessed the ability of ultrasonography to predict the presence and location of histologic lesions in the SI of cats. The clinical utility of this question is to help clinicians choose the best biopsy method for each patient. We did not aim to identify the best biopsy method for cats with chronic enteropathies. The choice of biopsy method is a multifaceted decision, based on invasiveness level, patient factors (eg, hypoalbuminemia), and diagnostic quality. An additional complicating factor is that IBD and SC-LSA may be present together, as a continuum, or may be present multifocally or diffusely.[24] From the clinician's perspective, the ideal biopsy method is the least invasive method that provides the most information. Endoscopic biopsies may be preferred to spare the patient from a surgical recovery, and for gross mucosal

TABLE 3   Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of ultrasonographic lesions in the jejunum to predict the presence of a histopathologic lesion in the duodenum or ileum

Small intestinal segment	Prevalence (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Duodenum (n = 123)	77.0% (69.0-84.3)	75.8% (65.9-84.0)	50.0% (30.6-69.4%)	83.7% (74.2-90.8)	37.8% (22.5-55.2)
Ileum (n = 89)	73.0% (63.0-81.9)	64.6% (51.8-76.1)	45.8% (25.6-67.2)	76.4% (63.0-86.8)	32.4% (17.4-50.5)

Prevalence of histopathologic lesions in each segment or layer is provided.
Abbreviations: 95% CI, 95% confidence interval; n, number of cases having both ultrasound and biopsy available for evaluation from each segment or layer.

TABLE 4 Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of ultrasonographic lesions in the muscularis layer to predict the presence of a histopathologic lesion in the same small intestinal segment's mucosa, submucosa, or both

Small intestinal segment and wall layer	Prevalence (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Duodenum					
Mucosa (n = 114)	73.0% (64.0-80.7)	33.7% (23.7-44.9)	80.6% (62.5-92.5)	82.4% (65.5-93.2)	31.3% (21.3-42.6)
Submucosa (n = 115)	17.0% (10.0-24.6)	52.6% (28.9-75.6)	75.0% (65.1-83.3)	29.4% (15.1-47.5)	88.9% (80.0-94.8%)
Jejunum					
Mucosa (n = 87)	87.0% (79.0-93.5)	69.7% (58.1-79.8)	54.5% (23.4-83.3)	91.4% (81.0-97.1)	20.7% (8.0-39.7)
Submucosa (n = 88)	38.0% (27.0-48.5)	78.8% (61.1-91.0)	41.8% (28.7-55.9)	44.8% (31.7-58.5)	76.7% (57.7-90.1)
Ileum					
Mucosa (n = 86)	71.0% (60.0-80.2)	52.5% (39.3-65.4)	80.0% (59.3-93.2)	86.5% (71.2-95.5)	40.8% (27.0-55.8)
Submucosa (n = 86)	28.0% (19.0-38.6)	50.0% (29.1-70.9)	61.3% (48.1-73.4)	33.3% (18.6-51)	76.0% (61.8-86.9)

Prevalence of histopathologic lesions in each segment or layer is provided.

Abbreviations: 95% CI, 95% confidence interval; n, number of cases having both ultrasound and biopsy available for evaluation from each segment or layer.

evaluation of lacteal dilatation, ulceration, and other lesions. From the pathologist's perspective, more tissue always gives a better diagnostic yield, making full thickness biopsy the preferred method. Because immunohistochemistry (IHC) and clonality studies are increasingly available, pathologists may be able to get more information from endoscopic biopsy specimens.[25] Without the availability of these tests, the diagnosis of IBD versus SC-LSA can be challenging without histologic evaluation of tissue deeper than the mucosa. The ideal balance in selecting a biopsy method is to make the decision on an individual patient basis, and before our study, the utility of ultrasonography to aid in this decision has not been evaluated.

We found that when assessing the ability of ultrasonography to predict the presence of histologic lesions in the feline SI in general terms (ie, without regard to which layer of the SI was affected), the test has a high PPV and a low NPV. The presence of an ultrasonographic lesion in any layer of the feline SI strongly supports that a histologic lesion will be present in at least 1 layer of that segment of SI. This finding is consistent with previous reports showing that histologic lesions are present in the SI in 96%-99% of cats with thickening of the SI wall on ultrasound examination and clinical signs of gastrointestinal disease.[26,27] The unique aspect of our study was to determine whether ultrasonography also could predict the location of histologic lesions. We assessed this possibility by evaluating the specific layer or layers of SI in which both ultrasonographic and histologic lesions were identified. Ultrasonographic lesions of the mucosal layer in all segments of the feline SI were strongly predictive of histologic lesions within the mucosa (PPV, 72.7%-100%). However, the presence of ultrasonographic lesions within the submucosal and muscularis layers was not predictive of histologic lesions in those layers. This information should assist clinicians in determining the best method and location of SI biopsy for an individual cat.

When interpreting measures of diagnostic utility of ultrasonography calculated from our data (sensitivity, specificity, PPV, NPV), the most clinically useful measures were PPV and NPV. The PPV and NPV are affected by the prevalence of disease in the population. Our study population had a high prevalence of disease, with histologic lesions in the SI of 132 of 169 cats (78.1%). A high prevalence of SI disease is

likely to be present in a clinical population of cats with clinical signs of gastrointestinal disease in which a clinician would choose to use ultrasonography to evaluate for the presence of SI lesions. A recent study showed a prevalence of SI disease of 96% in a clinical population of 300 cats with clinical signs of chronic gastrointestinal disease and ultrasonographic evidence of thickening of the SI.[27] However, as the prevalence of disease in a population decreases, the PPV will decrease whereas the NPV will increase. Therefore, the PPV and NPV in our study are not generalizable to a population with a lower prevalence of disease. For example, if ultrasonography is used to screen healthy animals or animals without signs of gastrointestinal disease, its ability to predict histologic disease will not be as strong and our calculated PPV and NPV values will not apply. For example, to demonstrate how prevalence can affect PPV and NPV, we calculated predictive values at various theoretical prevalence rates. For a histopathologic lesion in the duodenum (in any layer), with our calculated sensitivities and specificities, for a population with a prevalence of 30%, the PPV of ultrasonography would be 37.3% and the NPV would be 74.1%; for a population with a prevalence of 60%, the PPV would be 67.5% and NPV would be 44.9%, and for a population with a prevalence of 95%, the PPV would be 96.3% and NPV would be 6.1%. Similarly, for histopathologic lesions in the jejunum (any layer), with our calculated sensitivities and specificities, for a population with a prevalence of 30%, the PPV of ultrasonography would be 40.4% and the NPV would be 84.9%; for a population with a prevalence of 60%, the PPV would be 70.4% and NPV would be 61.6%, and for a population with a prevalence of 95%, the PPV would be 96.8% and NPV would be 11.2%. For histopathologic lesions in the ileum (any layer), with our calculated sensitivities and specificities, for a population with a prevalence of 30%, the PPV of ultrasonography would be 53.9% and the NPV would be 81.1%; for a population with a prevalence of 60%, the PPV would be 80.4% and NPV would be 55.1%, and for a population with a prevalence of 95%, the PPV would be 98.1% and NPV would be 8.8%.

For a population with high disease prevalence, as seen in cats with chronic gastrointestinal signs, the high PPV of an ultrasonographic lesion in any layer of the feline SI for the presence of a histologic lesion in any layer of the same segment of SI should help clinicians conclude

that intestinal biopsy should be recommended. If an ultrasonographic lesion is present, a histologic lesion is likely to be present in a population of cats suspected of having SI disease. This supports previous data regarding ultrasonographic thickening of the feline SI.[26,27] However, we evaluated the predictive value for any lesion, which included thickening, changes in echogenicity, hyperechoic bands, masses, and loss of normal layering structure. Any of these abnormalities is strongly predictive of histologic abnormality and their presence should warrant a recommendation of SI biopsy. In contrast, ultrasonographic lesions in any SI layer have low NPV and low sensitivity for histologic lesions. Therefore, the lack of an ultrasonographic lesion does not preclude the presence of a histologic lesion, as previously reported.[28,29] In a patient with a high clinical suspicion of SI disease, SI biopsy still may be indicated when no ultrasonographic change is present.

The ability of ultrasonography to predict the location of a histologic lesion within the layers of the SI wall may be useful to the clinician in deciding whether to recommend endoscopic biopsy versus full-thickness biopsy. Although endoscopic biopsies are less invasive, they may be insufficient for differentiating IBD from small-cell LSA in cats.[2] One advantage of full-thickness biopsy is that it allows for identification of a transmural lymphocytic infiltrate, which is highly supportive of LSA.[2,6] Our results showed a high PPV for mucosal ultrasonographic lesions to predict mucosal histologic lesions but a low PPV for submucosal or muscularis ultrasonographic lesions to predict histologic lesions in those layers. Ultrasonographic lesions in the submucosa or muscularis do not predict transmural disease and are not necessarily an indication for full-thickness biopsy. Furthermore, ultrasonographic lesions of the muscularis layer were strongly predictive of histologic lesions of the mucosal layer (PPV, 82.4%-91.4%) but not of the submucosal layer (PPV, 29.4%-44.8%). Our findings are in contrast to a prior study that found that ultrasonographic thickening of the muscularis layer was associated with histologic infiltrate of the mucosa and submucosa.[4] However, in that study, as in our study, ultrasonographic thickening of the muscularis layer was not associated with histologic infiltrate of the muscularis layer.[4] It has been suggested that thickening of the muscularis layer can be caused by muscular hypertrophy or muscular shortening, rather than by cellular infiltration.[4] The NPV for ultrasonographic lesions in the submucosal and muscularis layers was relatively high (66.2%-92.6%) indicating that the absence of ultrasonographic change in these layers suggests the absence of transmural disease.

Another limitation to endoscopic biopsy in the diagnosis of SC-LSA is the segmental nature of the disease. Cats with SC-LSA in 1 segment of SI and IBD in another segment commonly are reported in the veterinary literature, with segmental disease reported in as many as 33% of cats.[2,7,27] Endoscopic jejunal biopsy beyond the proximal jejunum in cats typically is not possible because of limitations of the equipment. Endoscopic ileal biopsy in cats can be technically challenging and may require blind biopsy without direct visualization of the mucosa.[7] The most common site of SC-LSA in the feline SI is the jejunum or ileum, highlighting a major limitation of endoscopic biopsy.[5,11,24] When evaluating the ability of ultrasonography to predict histologic disease of the jejunum in our study, we found a high PPV and low NPV when

considering lesions of any layer of the jejunum. Therefore, an abnormality on ultrasound examination is strongly suggestive of histologic disease in the jejunum, whereas the lack of an ultrasonographic lesion does not preclude the presence of a histologic lesion. When examining the small number of cats in our population in which the jejunum was the only segment of SI with an ultrasonographic abnormality, 62.9% had a histologic lesion in the duodenum or ileum. Regardless of ultrasound examination findings, it would be difficult to suggest that jejunal biopsy is unnecessary in an individual patient based on the results of our data. However, the frequency in which the jejunum is the sole site of small-cell LSA in not known. Although many cats with jejunal ultrasonographic lesions also had duodenal (83.7%) or ileal (76.4%) histologic lesions, we did not assess whether the histopathology was the same in all 3 segments. Our study was not designed to answer the question of whether endoscopic biopsy is sufficient to diagnose SC-LSA in cats. However, previous work suggesting that endoscopic biopsy is insufficient evaluated only gastric and duodenal biopsy specimens and did not utilize IHC or PCR for antigen receptor rearrangements (PARR) to confirm diagnoses. Combining duodenal and ileal sampling may increase the yield of endoscopic biopsy.[7] Utilization of IHC and PARR on duodenal endoscopic biopsy samples may further improve the sensitivity of endoscopic biopsy.[25]

Our study had several limitations owing to its retrospective design. It is unlikely that the areas of SI sampled correlated directly with the region of ultrasonographic abnormality in all cases. Although this makes it impossible to say there is a direct correlation between the ultrasonographic and histologic lesions, this situation accurately represents what happens in a clinical setting. Not all cats had histology samples collected from all 3 SI segments, creating a potential source of bias whereby segments that were biopsied were more likely to have gross or ultrasonographic change. Necropsy cases were included in our study to decrease selection bias for cats with jejunal ultrasonographic lesions. However, necropsy histology sample quality for gastrointestinal samples may be compromised because of autolysis, predominantly in the mucosal layer. The majority of diseased cats (79.5%) in our study had inflammation as their primary histopathologic change, whereas only 28.6% had LSA. This finding is in contrast to other studies in which the proportion of cats with LSA was 40%-80%.[4,6,25,27] The number of cats with LSA may be falsely decreased in our study, because the diagnoses were made purely on histologic evaluation, with final diagnosis determined by a single pathologist. The effect of inter-pathologist variation must also be considered. Advanced diagnostic tests (IHC and PARR) were not performed to distinguish IBD from SC-LSA, which may have led to undiagnosed emerging SC-LSA in some of the cats. However, our goal was not to find associations between specific ultrasonographic lesions and specific histopathologic lesions, and thus this limitation does not change our conclusions. Although other studies evaluating the utility of ultrasonography in the diagnosis of SI disease in cats have assessed a specific ultrasonographic finding of increased thickness, we chose to include any other ultrasonographic lesions that might be associated with disease, such as abnormal wall echogenicity (eg, hyperechoic mucosa, hyperechoic mucosal band) and wall masses.[4,26,27] Although this method did not allow us to evaluate

the diagnostic utility of specific lesions, it does provide a clinically relevant assessment of the utility of any ultrasonographic abnormality that may be associated with SI disease.

In summary, our data show that cats with SI ultrasonographic lesions are likely to have SI histologic lesions. Most cats with SI mucosal ultrasonographic lesions will have mucosal histologic lesions. Endoscopic mucosal biopsy should effectively identify these lesions. Mucosal ultrasonographic lesions in the jejunum are an indication for surgical biopsy, because the jejunum cannot be reliably evaluated using endoscopy. However, SI submucosal and muscularis ultrasonographic lesions are not predictive of histologic disease in the submucosal and muscularis layers, suggesting that full-thickness biopsy may not be essential to obtain a diagnosis in cats with ultrasonographic submucosal or muscularis lesions, or both. Cats lacking ultrasonographic lesions in the submucosal and muscularis layers rarely have histologic lesions in those layers, and these cats may be good candidates for endoscopic SI biopsy. Although the decision to recommend SI biopsy and which biopsy technique (endoscopic versus full-thickness) to recommend is multifactorial, our data may help guide recommendations in an individual cat.

ACKNOWLEDGMENT

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

ORCID

Talia Guttin https://orcid.org/0000-0002-4734-4623
Mark P. Rondeau https://orcid.org/0000-0002-1703-152X

REFERENCES

1. Baez JL, Hendrick MJ, Walker LM, Washabau RJ. Radiographic, ultrasonographic, and endoscopic findings in cats with inflammatory bowel disease of the stomach and small intestine: 33 cases (1990-1997). *J Am Vet Med Assoc*. 1999;215(3):349-354.
2. Evans SE, Bonczynski JJ, Broussard JD, et al. Comparison of endoscopic and full-thickness biopsy specimens for diagnosis of inflammatory bowel disease and alimentary tract lymphoma in cats. *J Am Vet Med Assoc*. 2006;229(9):1447-1450.
3. Lingard AE, Briscoe K, Beatty JA, et al. Low-grade alimentary lymphoma: clinicopathological findings and response to treatment in 17 cases. *J Feline Med Surg*. 2009;11:692-700.
4. Zwingenberger AL, Marks SL, Baker TW, et al. Ultrasonographic evaluation of the muscularis propria in cats with diffuse small intestinal lymphoma or inflammatory bowel disease. *J Vet Intern Med*. 2010;24(2):289-292.
5. Kleinschmidt S, Harder J, Nolte I, et al. Chronic inflammatory and non-inflammatory diseases of the gastrointestinal tract in cats: diagnostic advantages of full-thickness intestinal and extra-intestinal biopsies. *J Feline Med Surg*. 2010;12:97-103.
6. Kiupel M, Smedley RC, Pfent C, et al. Diagnostic algorithm to differentiate lymphoma from inflammation in feline small intestinal biopsy samples. *Vet Pathol*. 2011;48(1):212-222.
7. Scott KD, Zoran DL, Mansell J, et al. Utility of endoscopic biopsies of the duodenum and ileum for diagnosis of inflammatory bowel disease and small cell lymphoma in cats. *J Vet Intern Med*. 2011;25:1253-1257.
8. Fondacaro JV, Richter KP, Carpenter JL, et al. Feline gastrointestinal lymphoma: 67 cases (1988-1996). *Eur J Comp Gastroenterol*. 1999;4(2):5-11.
9. Day MJ, Bilzer T, Mansell J, et al. Histopathological standards for the diagnosis of gastrointestinal inflammation in endoscopic biopsy samples from the dog and cat: a report from the World Small Animal Veterinary Association Gastrointestinal Standardization Group. *J Comp Pathol*. 2008;138:S1-S43.
10. Casamian-Sorrosal D, Willard MD, Murray JK, et al. Comparison of histopathologic findings in biopsies from the duodenum and ileum of dogs with enteropathy. *J Vet Intern Med*. 2010;24:80-83.
11. Daniaux LA, Laurenson MP, Marks SL, et al. Ultrasonographic thickening of the muscularis propria in feline small intestinal small cell T-cell lymphoma and inflammatory bowel disease. *J Feline Med Surg*. 2014;16(2):89-98.
12. Kleinschmidt S, Meneses F, Nolte I, Hewicker-Trautwein M. Retrospective study on the diagnostic value of full-thickness biopsies from the stomach and intestines of dogs with chronic gastrointestinal disease symptoms. *Vet Pathol*. 2006;43:1000-1003.
13. Procoli F, Motskula PF, Keyte SV, et al. Comparison of histopathologic findings in duodenal and ileal endoscopic biopsies in dogs with chronic small intestinal enteropathies. *J Vet Intern Med*. 2013;27:268-274.
14. Washabau RJ, Day MJ, Willard MD, et al. Endoscopic, biopsy, and histopathologic guidelines for the evaluation of gastrointestinal inflammation in companion animals. *J Vet Intern Med*. 2010;24:10-26.
15. Leib MS, Larson MM, Panciera DL, et al. Diagnostic utility of abdominal ultrasonography in dogs with chronic vomiting. *J Vet Intern Med*. 2010;24:803-808.
16. Leib MS, Larson MM, Grant DC, et al. Diagnostic utility of abdominal ultrasonography in dogs with chronic diarrhea. *J Vet Intern Med*. 2012;26:1288-1294.
17. Penninck DG, Webster CRL, Keating JH. The sonographic appearance of intestinal mucosal fibrosis in cats. *Vet Radiol Ultrasound*. 2010;51(4):458-461.
18. Di Donato P, Penninck D, Pietra M, et al. Ultrasonographic measurement of the relative thickness of intestinal wall layers in clinically healthy cats. *J Feline Med Surg*. 2014;16(4):333-339.
19. Winter MD, Londono L, Berry CR, Hernandez JA. Ultrasonographic evaluation of relative gastrointestinal layer thickness in cats without clinical evidence of gastrointestinal tract disease. *J Feline Med Surg*. 2014;16:118-124.
20. Diana A, Pietra M, Guglielmini C, et al. Ultrasonographic and pathologic features of intestinal smooth muscle hypertrophy in four cats. *Vet Radiol Ultrasound*. 2003;44(5):566-569.

21. Sutherland-Smith J, Penninck DG, Keating JH, Webster CL. Ultrasonographic intestinal hyperechoic mucosal striations in dogs are associated with lacteal dilation. *Vet Radiol Ultrasound*. 2007;48:51-57.

22. Gaschen L. Ultrasonography of small intestinal inflammatory and neoplastic diseases in dogs and cats. *Vet Clin North Am Small Anim Pract*. 2011;41:329-344.

23. Tucker S, Pennick DG, Keating JH, Webster CRL. Clinicopathological and ultrasonographic features of cats with eosinophilic enteritis. *J Feline Med Surg*. 2014;16:950-956.

24. Moore PF, Rodriguez-Bertos A, Kass PH. Feline gastrointestinal lymphoma: mucosal architecture, immunophenotype, and molecular clonality. *Vet Pathol*. 2012;49(4):658-668.

25. Sabattini S, Bottero E, Turba ME, et al. Differentiating feline inflammatory bowel disease from alimentary lymphoma in duodenal endoscopic biopsies. *J Small Anim Pract*. 2016;57(8):396-401.

26. Norsworthy GD, Estep JS, Kiupel M, et al. Diagnosis of chronic small bowel disease in cats: 100 cases (2008-2012). *J Am Vet Med Assoc*. 2013;243:1455-1461.

27. Norsworthy GD, Estep JS, Hollinger C, et al. Prevalence and underlying causes of histologic abnormalities in cats suspected to have chronic small bowel disease: 300 cases (2008-2013). *J Am Vet Med Assoc*. 2015;247:629-635.

28. Rudorf H, van Schaik G, O'Brien RT, et al. Ultrasonographic evaluation of the thickness of the small intestinal wall in dogs with inflammatory bowel disease. *J Small Anim Pract*. 2005;46(7):322-326.

29. Gaschen L, Kircher P, Stüssi A, et al. Comparison of ultrasonographic findings with clinical activity index (CIBDAI) and diagnosis in dogs with chronic enteropathies. *Vet Radiol Ultrasound*. 2008;49(1):56-64.