

# Ultrasonographic Evaluation of the Muscularis Propria in Cats with Diffuse Small Intestinal Lymphoma or Inflammatory Bowel Disease

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**Background:** An ultrasonographic pattern of thickened muscularis propria in the small intestine and lymphadenopathy have been associated with gastrointestinal lymphoma and inflammatory bowel disease (IBD) in cats.

**Objectives:** To investigate the association of these imaging biomarkers with IBD and lymphoma in cats.

**Animals:** One hundred and forty-two cats with a histologic diagnosis of normal small intestine (SI) ( $n = 56$ ), lymphoma ( $n = 62$ ), or IBD ( $n = 24$ ).

**Methods:** Retrospective case review. Pathology records from 1998–2006 were searched for cats with a diagnosis of normal, IBD, or lymphoma, an ultrasonographic examination <28 days before surgery, and without ultrasonographic evidence of a mass. Multinomial regression analysis was used to determine the association of imaging biomarkers with disease status.

**Results:** Cats with thickening of the muscularis propria detected by ultrasonographic examination were more likely to have lymphoma compared with normal SI cats (odds ratio [OR] = 4.0, 95% confidence interval [95% CI] 1.2–13.1,  $P = .021$ ) and those with IBD (OR = 18.8, 95% CI 2.2–162.7,  $P = .008$ ). Histologic samples of cats with muscularis propria thickening were more likely to have disease infiltrates in both the mucosal and submucosal layers (OR = 8.1, 95% CI 1.7–38.4,  $P = .008$ ) than cats with normal SI. Cats with ultrasonographic evidence of lymphadenopathy were more likely to have a diagnosis of lymphoma (OR = 44.9, 95% CI 5.1–393.0,  $P = .001$ ) or IBD (OR = 10.8, 95% CI 1.1–106.3,  $P = .041$ ) than normal SI. Fifty-six of 62 cats had confirmed or presumptive diagnosis of diffuse T-cell lymphoma.

**Conclusions and Clinical Relevance:** Older cats with muscularis layer thickening are more likely to have T-cell lymphoma than IBD. The ultrasonographic pattern is associated with histologic infiltrates in the mucosal and submucosal layers of small intestine. Lymphadenopathy is associated with lymphoma or IBD.

**Key words:** Anatomy and pathology; Feline; Lymphosarcoma; Oncology diagnosis.

The normal wall layers of the small intestine, including mucosa, submucosa, and muscularis, are visible on routine ultrasonographic examinations with high-frequency transducers.<sup>1</sup> Thickening of the muscularis propria is associated with diffuse infiltrative bowel disease such as lymphoma or inflammatory bowel disease (IBD) in cats (Fig 1).<sup>2</sup> Muscularis propria thickening is reported in 2 cats with IBD, 1 proximal to a small intestinal mass, and 1 proximal to an obstruction. The cause of this thickening was determined to be muscular hypertrophy.<sup>3</sup>

Most descriptions of ultrasonographic features of gastrointestinal lymphoma in cats are of lymphomatous masses.<sup>4–7</sup> Both T-cell and B-cell lymphoma are described in studies using immunohistochemistry.<sup>8–10</sup> There was no distinction made between diffuse and focal disease in these reports. An epitheliotropic T-cell lymphoma has been described with diffuse, mild ultrasonographic findings with or without a mass, or

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## Abbreviations:

IBD	inflammatory bowel disease
SI	small intestine

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with no ultrasonographic abnormalities.<sup>11</sup> Studies of IBD or lymphoma in cats have demonstrated a predominance of the T-cell phenotype, arising from the mucosal-associated lymphoid tissue.<sup>12,13</sup> This is described as mucosal or transmural lymphoma,<sup>13</sup> which suggests a centrifugal progression of disease, and that there is a disease state before presence of a mass. Thickening of the small intestinal wall is not a good predictor of IBD in dogs,<sup>14</sup> while thickening and loss of layering is highly predictive of focal intestinal neoplasia in dogs and cats.<sup>6,15</sup> The challenge lies in detecting lymphoma or IBD in cats without ultrasonographic loss of small intestinal layering indicating a mass, using new biomarkers. IBD and T-cell lymphoma could have different ultrasonographic findings than those portrayed for the more commonly described gastrointestinal lymphoma masses.

The goals of this study were to determine the association between muscularis propria thickening and disease type in cats with lymphoma, IBD, or normal SI; to determine the association of ultrasonographic and histopathologic abnormalities; and to characterize the type of lymphoma seen with this pattern.

## Materials and Methods

Records of the Veterinary Medical Teaching Hospital at the University of California, Davis, from 1998 to 2006 were searched to

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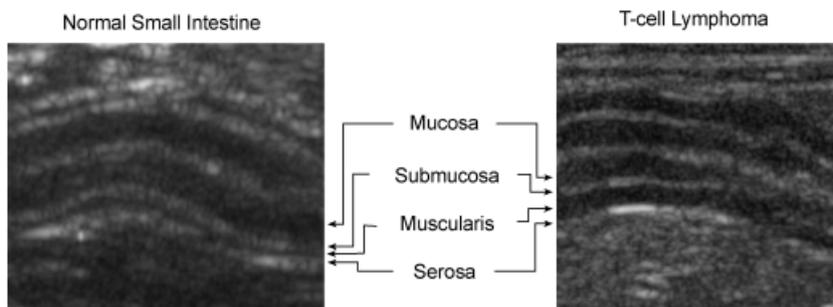
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**Fig 1.** Sagittal ultrasound images of feline normal small intestine and T-cell lymphoma with a linear 8–12 MHz transducer. The mucosal, submucosal, muscularis, and serosal layers are indicated with arrows in the far field. Compared with the normal image on the left, the muscularis propria is thickened in the cat affected with T-cell lymphoma.

identify cats that had a full-thickness intestinal biopsy and an abdominal ultrasonographic examination in the 28 days before biopsy. Pathology reports were evaluated to identify cats with a diagnosis of lymphoma, IBD, or normal SI. Diagnoses were limited to disease in the duodenum, jejunum, and ileum. Immunohistochemical diagnosis of lymphoma type was recorded in those cats with lymphoma when available. Cats with positive clonality assays for the T-cell receptor gamma rearrangement and compatible histopathological morphology were given a presumptive diagnosis of T-cell lymphoma.

IBD can appear morphologically similar to lymphoma as both conditions result from expansion of T-cell populations from the mucosal-associated lymphoid tissue of the small intestine.<sup>13</sup> For this reason, the samples originally classified as IBD ( $n = 34$ ) were tested to identify clonal or oligoclonal populations that would change the diagnosis to lymphoma. Histopathological slides of cats with reported diagnoses of IBD were reviewed by a veterinary pathologist (PFM) and tissue blocks were analyzed with clonality assays,<sup>13</sup> to confirm their diagnosis or to reclassify them as lymphoma. Presence of abnormal cells in the mucosa/lamina propria, submucosa, and muscularis propria was recorded where available. Samples of cats with normal SI were not evaluated for clonality. Ultrasound reports and available images were reviewed for presence of muscularis propria thickening of the small intestine, and presence of mesenteric lymphadenopathy, including enlarged or hypoechoic lymph nodes. Muscularis propria thickening was defined as being present when the width of the muscularis interna and externa of the small intestine was greater than half the thickness of the submucosal layer. Those with loss of small intestinal layering, a mass, or more than 1 tumor type were not included.

Statistical analysis included analysis of variance, Pearson's  $\chi^2$ , and multinomial logistic regression. Normal SI and IBD groups were used as the referent groups in separate multinomial logistic regression analyses. A  $P$  value of  $< .05$  was considered significant, and odds ratios (OR) were generated for the regression models.

## Results

### Study Group

One hundred and forty-two cats with a histological diagnosis of normal SI, lymphoma, or IBD determined via full-thickness biopsy, and that had an ultrasonographic examination within 28 days before biopsy, were identified. Of these, 10 samples from the original 34 (29%) in the IBD group were reclassified as lymphoma, based on clonality studies. The total number of samples from each

group after reclassification was normal SI ( $n = 56$ ), lymphoma ( $n = 62$ ), and IBD ( $n = 24$ ).

Cats with lymphoma were significantly older ( $12.6 \pm 3.2$ ) than those with IBD ( $8.9 \pm 5.1$ ) or normal SI ( $8.6 \pm 3.6$ ) ( $P < .001$ ). Therefore age was included as a confounding variable in all logistic regression models.

Forty-five cats with original diagnoses of lymphoma had been confirmed with clonality, and an additional 12 were confirmed during this study, for a total of 57 of 62.

Forty-four cats had T-cell lymphoma confirmed by immunohistochemistry, and 12 had presumptive diagnosis of T-cell lymphoma with positive T-cell receptor  $\gamma$  clonality and compatible histopathological morphology. Fifty-six of 57 (98.2%) cats had T-cell lymphoma, and one had non-T, non-B cell lymphoma. No cats were diagnosed with B-cell lymphoma.

### Relationship of Ultrasound Findings to Disease Group

Ultrasonographic thickening of the muscularis propria was seen in 7 of 56 cats with normal SI (12.5%), 30 of 62 cats with lymphoma (48.4%), and 1 of 24 cats with IBD (4.2%). Cats with a pattern of thickening of the muscularis propria on ultrasound exam were more likely to have lymphoma compared with normal SI cats (OR = 4.0, 95% confidence interval [95% CI] 1.2–13.1,  $P = .021$ ) and those with IBD (OR = 18.8, 95% CI 2.2–162.7,  $P = .008$ ).

Lymphadenopathy was reported in 1 of 56 normal SI cats (2%), 29 of 62 cats with lymphoma (47%), and 4 of 24 cats with IBD (17%). Cats with ultrasonographic evidence of lymphadenopathy were more likely to have a diagnosis of lymphoma (OR = 44.9, 95% CI 5.1–393.0,  $P = .001$ ) or IBD (OR = 10.8, 95% CI 1.1–106.3,  $P = .041$ ) than normal SI. Cats with lymphoma did not have significantly more lymphadenopathy than those with IBD ( $P = .09$ ).

Of the cats with lymphoma, 16 of 62 (26%) had both muscularis propria thickening and lymphadenopathy. Only 1 of 24 (4%) cats with IBD and 1 of 56 (2%) normal SI cats had both muscularis thickening and lymphadenopathy. Cats with lymphoma were more likely to have both muscularis thickening and lymphadenopathy compared with the IBD group ( $P = .024$ ) and the normal SI cats ( $P < .001$ ).

### ***Relationship of Ultrasound Findings to Histopathology***

The biomarkers were compared with the number of small intestinal wall layers that contained pathologic infiltrates to determine whether there was an association between imaging findings and severity of disease. Less severe infiltrates were confined to the mucosal layer, transmural infiltrates involved the mucosal and submucosal layers, and severe transmural infiltrates involved all three layers.

Samples of cats with muscularis propria thickening were more likely to have transmural infiltrates (OR = 8.1, 95% CI 1.7–38.4,  $P = .008$ ) than cats with normal SI. Muscularis propria thickening was not significantly associated with severe transmural disease ( $P = .19$ ), or with mucosal disease ( $P = .58$ ).

Samples of cats with lymphadenopathy on ultrasonographic examination were more likely to have transmural infiltrates (OR = 24.3, 95% CI 2.4–251.3,  $P = .007$ ) and severe transmural infiltrates (OR = 80.1, 95% CI 7.9–812.8,  $P < .001$ ) than those with normal SI. Lymphadenopathy was not significantly associated with mucosal infiltrates ( $P = .14$ ).

### **Discussion**

Our findings indicate ultrasonographic thickening of the muscularis propria of the small intestine is significantly associated with small intestinal lymphoma (98% T-cell) in cats, and lymphadenopathy is associated with both lymphoma and IBD. These imaging biomarkers were also evaluated for prediction of the number of intestinal layers affected by the infiltrative disease. Muscular layer thickening on ultrasonographic examination indicated higher odds of having disease present in the mucosal and submucosal layers, and lymphadenopathy was associated with presence of disease in the mucosal and submucosal, or mucosal, submucosal, and muscularis propria. Disease confined to the mucosa and lamina propria was not associated with ultrasonographic changes. Muscularis propria thickening was not associated with IBD in this population of cats.

By the histopathological classification system of mucosal (confined to the mucosa) and transmural (mucosa and additional layers) disease, ultrasonographic abnormalities are predictive of transmural disease. These results confirm the clinically observed association between the ultrasonographic pattern of muscularis thickening and lymphoma, and demonstrate the increased odds of T-cell lymphoma when muscularis thickening and lymphadenopathy are observed.

This study was designed to evaluate ultrasonographic features of small intestinal disease in cats without a mass lesion that would indicate neoplasia. To date, diffuse small intestinal IBD and lymphoma must be differentiated by biopsy. As our collective experience with ultrasonographic examination has increased, and as machines have improved technically in contrast and spatial resolution, more subtle changes in bowel wall layering have been noted. Alterations in layer thickness may indicate disease affecting that segment or layer of small

intestine. Secondary changes of enlarged mesenteric lymph nodes can indicate inflammation, reactivity, or metastatic disease from the small intestine. These biomarkers were evaluated as predictors of the presence of small intestinal lymphoma or IBD in cats.

The majority of cases of lymphoma were confirmed by immunohistochemistry and clonality assays. IBD can appear very similar to T-cell lymphoma on routine histopathology. This is underlined by our results in which 29% of cats that had originally been diagnosed with IBD actually had lymphoma. When these diagnostic tests are not performed, cats with lymphoma or IBD could be misclassified. This may be a factor in the clinical observation that thickened muscularis propria is associated with IBD, which was not supported in this population of cats.

The proposed pathophysiology of T-cell lymphoma is that it begins as a clonal population of malignant lymphocytes that remains in the mucosal region for a period of time because of continued expression of mucosal homing integrins and chemokine receptors.<sup>16</sup> Eventually, it may progress from a mucosal to a transmural form. In this case, changes visible ultrasonographically predict the presence of the more advanced stages of the disease after it has progressed from the mucosa through the outer wall layers. Muscularis thickening gives increased odds of disease to the depth of the submucosa (transmural), and lymph node enlargement indicates submucosa and muscularis involvement (severe transmural). These findings could help to stage or grade the extent of the lymphoma.

The visible changes in the lymph nodes and muscularis propria might not be directly caused by increased numbers of lymphocytes in these areas; however, they can serve as biomarkers for the disease. Only 43% of the cats with microscopically visible disease in the muscularis had muscularis propria thickening on ultrasonographic examination. Some of the visible changes could be because of infiltration, but in other cases it could be a function of muscular hypertrophy or muscular shortening causing the appearance of layer thickening. Discrepancy between ultrasound and biopsy site could also cause false negative histopathology findings in the muscularis propria.

Muscularis thickening can be seen in apparently healthy animals, and in those with masses involving the small intestine. Muscularis thickening is not pathognomonic for lymphoma in cats, but the odds of this disease being present are greater when the pattern is observed. Full-thickness biopsy and assessment of clonality should be considered if clinically warranted. In cases where clinical signs are compatible with small intestinal lymphoma, there is no ultrasonographically visible mass, and tissue sampling is not possible, this finding could form the basis for initiating therapy for lymphoma.

Although IBD and T-cell lymphoma are extensive segmental or diffuse diseases, the random sampling nature of both ultrasound imaging and surgical biopsy in this study could affect the number of false negative histological samples and ultrasound findings. The image records did not have sufficient uniformity in position and location to enable direct measurements of the wall layers. Despite this, the study had enough power to detect

significant associations between biomarkers, disease status, and depth of penetration of disease. These findings confirm the clinical suspicion of an association of muscularis propria thickening with lymphoma, and increase the diagnostic yield of the ultrasonographic examination. Measurement metrics on standardized-ultrasound image sets may help to further define the wall layering changes in future studies.

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