Accepted Manuscript

Title: Maximizing the diagnostic utility of endoscopic biopsy in dogs and cats with gastrointestinal disease

Author: Albert E. Jergens, Michael D. Willard, Karin Allenspach

PII: S1090-0233(16)30030-2
DOI: http://dx.doi.org/doi: 10.1016/j.tvjl.2016.04.008
Reference: YTVJL 4801

To appear in: *The Veterinary Journal*

Accepted date: 14-4-2016

Please cite this article as: Albert E. Jergens, Michael D. Willard, Karin Allenspach, Maximizing the diagnostic utility of endoscopic biopsy in dogs and cats with gastrointestinal disease, *The Veterinary Journal* (2016), http://dx.doi.org/doi: 10.1016/j.tvjl.2016.04.008.

Commissioned Review

Maximizing the diagnostic utility of endoscopic biopsy in dogs and cats with gastrointestinal disease

Albert E. Jergens[a,*], Michael D. Willard[b], Karin Allenspach[c]

[a]*Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IA, 50010, USA*
[b]*Department of Small Animal Clinical Sciences, College of Veterinary Medicine, Texas A and M University, College Station, TX, 77843, USA*
[c]*Royal Veterinary College, University of London, Hawkshead Lane, North Mymms, AL9 7PT, UK*

* Corresponding author. Tel.: +1-515 460 1205.
E-mail address: ajergens@iastate.edu (A. Jergens).

Highlights:

- Collect endoscopic biopsies (including ileum) regardless of mucosal appearance
- Operator experience and biopsy number impact the quality of endoscopic specimens
- Large cup biopsy forceps yield the best diagnostic tissue specimens
- Histopathologic guidelines for endoscopic biopsy are published and are evolving

Abstract

Flexible endoscopy has become a valuable tool for the diagnosis of many small animal gastrointestinal (GI) diseases, but the techniques must be performed carefully so that the results are meaningful. This article reviews the current diagnostic utility of flexible endoscopy, including practical/technical considerations for endoscopic biopsy, optimal instrumentation for mucosal specimen collection, the correlation of endoscopic indices to clinical activity and to histopathologic findings, and new developments in the endoscopic diagnosis of GI disease. Recent studies have defined endoscopic biopsy guidelines for the optimal number and quality of diagnostic specimens from different regions of the gut. They also have shown the value of ileal biopsy in the diagnosis of canine and feline chronic enteropathies, and have demonstrated the

utility of endoscopic biopsy specimens beyond routine hematoxylin and eosin histopathological analysis, including their use in immunohistochemical, microbiological, and molecular studies.

Keywords: Gastrointestinal endoscopy; Biopsy; Histopathology; Inflammatory bowel disease; Small animal

Introduction

After its introduction into clinical veterinary practice over 40 years ago, flexible endoscopy rapidly became a valuable tool for the diagnosis of many small animal gastrointestinal (GI) diseases. The clinical indications and practical considerations for performing GI endoscopic procedures have been extensively reviewed elsewhere (Simpson and Else, 1987; Willard, 2001; Zoran, 2001; Mansell and Willard, 2003; Washabau et al., 2010; Table 1). Flexible endoscopy provides non-invasive assessment of the GI mucosa and allows targeted collection of tissues, cells, and/or fluids for analysis. Tissue samples can be helpful in establishing a definitive diagnosis, prognosis, and therapeutic approach to many infiltrative chronic enteropathies (CE) in dogs and cats. Sequential biopsies might be useful in monitoring the response to therapy of select GI diseases. However, endoscopic biopsy cannot be used as a panacea for diagnosing all GI disorders, especially when appropriate anthelmintic, dietary, and antimicrobial trials have not been performed first in an effort to attenuate/resolve GI signs.

There is controversy regarding the value of endoscopic biopsy for diagnosing select GI diseases, especially feline alimentary small cell lymphoma (Evans et al., 2006), because endoscopic biopsy specimens are small and delicate compared to surgically obtained tissue samples. Other factors contributing to frustration with endoscopic biopsy are related to

inadequate operator experience (Slovak et al., 2014), poor endoscopic biopsy techniques (Willard et al., 2008), the need for precise tissue processing of samples, and non-uniform histopathologic grading criteria, all of which negatively impact correct diagnosis (Day et al., 2008).

This article reviews the current diagnostic utility of flexible endoscopy, including practical and technical considerations for endoscopic biopsy, optimal instrumentation for mucosal specimen collection, correlation of endoscopic indices to clinical activity and to histopathologic findings, and new developments in the endoscopic diagnosis of GI disease.

Practical considerations for endoscopic biopsy

Endoscopic biopsy of the GI tract has advantages and disadvantages. First, direct assessment of mucosal lesions undetectable from the serosal surface allows targeted biopsy. Second, being able to obtain numerous tissue specimens over a 10 – 20 cm length of intestine is more likely to detect mucosal lesions that can be regionally patchy in distribution (e.g., lymphoma, histoplasmosis, pythiosis, granulomatous colitis; Casamian-Sorrosal et al., 2010; Scott et al., 2011). However, while useful for detecting morphologic or infiltrative disease, endoscopy cannot detect functional disorders of the GI tract (Table 2). Furthermore, histopathology is of minimal use in diagnosing some forms of CE (e.g., food-responsive and antimicrobial-responsive disorders) since clinical response to therapeutic intervention is the most relevant and obvious outcome measure.

The decision to perform endoscopic biopsy is generally made following integration of laboratory tests and diagnostic imaging and after the procedure has been discussed with the pet owner. Health status, procedural time, costs, and inherent risks/benefits should be considered. If endoscopic biopsy is deemed appropriate, then instrumentation for optimal specimen collection must be considered. Different alimentary organs require different sampling instruments and techniques for optimal results. For example, it is almost impossible (and rarely indicated) to obtain good tissue samples of the esophageal mucosa with a flexible endoscope unless a mass is present. In such instances, exfoliative cytopathology specimens (brush cytology) might be useful. Mucosal biopsy of the stomach, small intestine and colon is more commonly indicated and is best performed with pinch biopsy forceps. Localized lesions (e.g., ulcers, masses, strictures) are best approached by either biopsying the transition zone (which can be difficult) or acquiring abnormal and normal tissue immediately adjacent to the lesion. Even some generalized disorders (e.g., lymphangiectasia) can have focal abnormalities. Other mucosal disorders can be so generalized that random biopsies of the affected organs are sufficient (e.g., inflammatory bowel disease [IBD], diffuse gastritis, diffuse neoplasia, diffuse fungal infections). Lastly, the nature of the suspected lesion (superficial vs. deep mucosal disease) influences instrument selection and biopsy technique. Non-lymphomatous mass lesions suspected to be neoplastic are sometimes best sampled by repeated biopsies from the same site. This can allow the clinician to collect deeper, more representative tissue samples and avoid necrotic surface debris and superficial inflammatory cells that might confuse the diagnostic procedure.

Potential complications of this biopsy technique include mural perforation with the biopsy forceps and/or endoscopic insertion tube as it is advanced along the GI tract. Practically

speaking, complications associated with GI endoscopic biopsy are rare. Contraindications for endoscopic biopsy generally relate to anesthetic risks and include severe generalized debilitation, pre-existing cardio-pulmonary disease, severe hypoproteinemia (hypotension), and coagulopathy.

Technical considerations for endoscopic biopsy

There are a variety of technical considerations when endoscopic biopsy is required for diagnosis of GI disease. These considerations include: (1) the ongoing controversy as to whether endoscopy or surgery is the preferred technique for intestinal biopsy; (2) determining which alimentary sites to sample; (3) optimal selection of endoscopic instruments for specimen collection; and (4) post-procurement handling protocols for endoscopic specimens to maximize accurate histopathologic interpretation.

'How do I determine whether endoscopic biopsy vs. full-thickness (surgical) biopsy is best for my veterinary patient?'

The controversy surrounding endoscopic and surgical biopsy methods centers on the acquisition of quality mucosal samples from different sections of the GI tract. Surgical biopsy is transmural, containing all layers of the gut, and it allows access to the entire intestinal tract. Disadvantages include a more invasive procedure, longer anesthetic times with increased risks for debilitated animals, prolonged hospitalization, greater procedural costs, and inability to see mucosal lesions which prevent directed biopsy (Gieger, 2011). One recent study demonstrating the diagnostic utility of surgical biopsy in 43 cats with chronic GI signs showed that full-thickness biopsies were useful in the diagnosis of IBD (47%), low grade lymphoma (23%)

mucosal fibrosis (9%), gastritis (7%), lymphangiectasia (7%), and mast cell tumors (5%; (Kleinschmidt et al., 2010). Multi-organ inflammatory GI disease (IBD) is common in cats, simultaneously involving the intestines, liver (cholangitis), and/or pancreas, and laparotomy is required for diagnosis (Jergens, 2012). Lingard et al. (2009) diagnosed low-grade lymphoma in 17 cats by histological evaluation of surgical biopsies from multiple regions of the GI tract collected during exploratory laparotomy.

Endoscopic biopsy is a less invasive procedure, takes less time to perform in critical animals, and allows assessment of the mucosa to identify the best biopsy sites. The results obtained from endoscopic biopsy are correlated with clinical experience, operator expertise, and the acquisition of diagnostic biopsy specimens for histopathologic review (Slovak et al., 2014; Willard et al., 2001). This is especially true with duodenal biopsy specimens where marginal specimens were defined as having at least one villus plus subvillus lamina propria, and adequate specimens contained at least three villi with subvillus lamina propria that extended down to the muscularis mucosa. A correct diagnosis was most likely to be obtained in one study when six marginal or adequate biopsies of the feline stomach or duodenum were obtained by the endoscopist (Willard et al., 2008).

One disadvantage with endoscopic biopsy is that only gastric, duodenal, and colonic mucosa can routinely be biopsied. High jejunal tissue can sometimes be obtained, and ileal tissue can be obtained in most animals if the operator is experienced. However, even competent endoscopists cannot always sample the ileum. There are surprisingly few data comparing the accuracy of full-thickness vs. endoscopic biopsy for diagnosis of canine and feline GI disease.

Endoscopic biopsy specimens of the duodenum were considered inadequate vs. full-thickness biopsies for differentiating IBD from lymphoma in one study (Evans et al., 2006). However, endoscopic assessment of the duodenum was limited to 50% of the cats, and mucosal biopsy was performed blindly (with only three specimens obtained per cat) in 8/22 (28%) of the cats. Because none of the cats in this study had endoscopic biopsy of the ileum performed, malignant infiltrates in this organ could only be confirmed in full-thickness specimens obtained by laparoscopy, which could have biased the results and over-interpreted the diagnostic value of full-thickness vs. endoscopic mucosal biopsy for feline lymphoma. In another study, the probability of diagnosing alimentary lymphoma was greatest in cats undergoing laparotomy with multi-organ biopsy from all segments of the intestine and the mesenteric lymph nodes (Kleinschmidt et al., 2010). Importantly, comparative data describing endoscopic biopsy results from the different intestinal segments in the cats of this study was not provided.

To summarize, different clinical situations dictate a preference for surgical vs. endoscopic biopsy. Comparative studies documenting the superiority of one biopsy technique over the other have not been published. Biopsy specimens obtained from multiple intestinal segments, including the ileum, enhance the sensitivity for diagnosis of lymphoma and other GI diseases. Other clues for alimentary lymphoma in cats may include transmural intestinal thickening, disrupted wall layers, and intestinal mass lesions. (Gieger, 2011) Surgical biopsy may be indicated if involvement of the submucosa or muscularis layer is suspected, or when endoscopic biopsy findings fail to correlate with clinical features (Baez et al., 1999). Laparoscopy is another option for obtaining full-thickness samples from different sections of the intestine (Webb, 2008).

'When it is best to perform upper GI endoscopy, lower GI endoscopy or both upper and lower GI endoscopy procedures in a veterinary patient?'

Salient GI signs help to localize the disease and inform the clinician regarding which organs should be examined (Table 1). Gastroscopy is usually performed in conjunction with esophagoscopy and duodenoscopy, and is considered more sensitive than barium contrast studies for the diagnosis of gastric mucosal disorders. Standard enteroscopy of the small intestine allows evaluation/biopsy of the duodenum and sometimes the proximal jejunum and ileum. (Washabau et al., 2010) Ileoscopy necessitates colonoscopy. Animals with protein-losing enteropathy should usually undergo mucosal biopsy of the small intestine to determine the cause of enteric plasma protein loss. Abdominal ultrasound may demonstrate small intestinal hyperechoic mucosal striations suggestive of lymphatic dilation in dogs with intestinal lymphangiectasia. In one retrospective study involving 23 dogs, histopathologic lacteal dilation of endoscopic (*n*=13 dogs) and full-thickness (*n*=9 dogs) mucosal specimens was present in 96% of dogs with mucosal striations (Sutherland-Smith et al., 2007). Low serum folate and cobalamin concentrations are another indication for endoscopy and suggest a focal or diffuse mucosal disorder affecting absorption in the proximal (duodenal) and distal (ileum) small intestine, respectively. Colonoscopy is indicated in animals with chronic or recurrent large bowel diarrhea that do not respond to therapeutic trials for parasites, and those that have dietary-responsive colitis, or selected bacterial-mediated colitis, such as *Campylobacter jejuni*. Ileoscopy is performed in conjunction with upper GI endoscopy when a diffuse enteropathy (e.g., IBD, lymphoma, histoplasmosis, lymphangiectasia) is suspected and when colonic disease is complicated by systemic signs, such as anorexia and weight loss.

Proper veterinary patient preparation for GI endoscopy optimizes the assessment of mucosal abnormalities and biopsy technique. For upper GI endoscopic procedures, withholding food but not water overnight generally allows thorough evaluation of the esophagus, stomach, and proximal duodenum in most dogs and cats. For ileoscopy/colonoscopy, more complete cleansing of the mucosa is required, using tepid water enemas and polyethylene glycol laxative solutions (with electrolytes) to fully visualize all colonic mucosal regions (Richter and Cleveland, 1989).

Mucosal masses, friability, granularity, and ulcers or erosions are commonly associated with histopathologic abnormalities (Table 3; Fig. 1). Mucosal abnormalities may be focal, patchy, or diffuse in distribution and may involve one or more alimentary tract regions. In one early study, endoscopic examination of the stomach, duodenum, and colon in 58 dogs and 17 cats with histories of CE showed that normal endoscopic observations were often associated with normal histopathologic findings (Roth et al., 1990a). However, visualizing masses, increased granularity or friability, and ulcers/erosions was associated with increased cellularity within the lamina propria attributable to mucosal inflammation (49%) or neoplasia (23%). White speckles and spots on the duodenal mucosa plus hypoalbuminemia have been reported in dogs with intestinal lymphangiectasia (Garcia-Sancho et al., 2011; Larson et al., 2012). These same endoscopic variables (e.g., granularity, friability, ulcer/erosions, mass) have been associated with the severity of clinical (GI) signs (Jergens et al., 2003a, b; Allenspach et al., 2007; Garcia-Sancho et al., 2007; Heilmann et al., 2012; Heilmann et al., 2014), evidence of mucosal healing (Allenspach et al., 2007; Garcia-Sancho et al., 2007), histopathologic interpretation (Roth et al.,

1990a; Jergens et al., 1992; Allenspach et al., 2007; Garcia-Sancho et al., 2007; Larson et al., 2012), and prognostic outcome (Allenspach et al., 2007) in separate clinical trials.

We have observed, as described in humans, that the observer variation for graded characteristics (e.g., mucosal hyperemia – is it pale, pink, or red?) is high, while that for discontinuous variables (i.e., presence or absence of erosions) is generally low (Baron et al., 1964). The discrepancy between mucosal hyperemia and the presence of histopathologic abnormalities in dogs with chronic enteropathy has been previously reported (Roth et al., 1990a). Operator experience plays an important role in endoscopic mucosal assessment; novice endoscopists more likely to miss mucosal lesions or misinterpret normal vs. abnormal mucosa (Roth et al., 1990a; Slovak et al., 2014).

New advanced imaging techniques, including magnification endoscopy, dye-based and dye-less chromoendoscopy, and endomicroscopy, provide real-time insights into the ultrastructural assessment of mucosal inflammation and dysplasia in humans (Rath et al., 2015). Chromoendoscopy permits detailed evaluation of the mucosal surface while other modalities (i.e., endocytoscopy and confocal endomicroscopy) go deeper within the intestinal wall to visualize the submucosal architecture and single cells. In vivo confocal endomicroscopy has been recently used for cellular and subcellular imaging of gastric (Sharman et al., 2014) and intestinal (Sharman et al., 2013) topography in healthy dogs.

'Which endoscopic instruments work best to sample the GI mucosa and how many biopsy samples do I need to make a diagnosis?'

Endoscopic instruments for sampling GI mucosa include pinch biopsy forceps and guarded cytology brushes. Flexible pinch forceps are most commonly used to obtain mucosal specimens from the GI tract. These small flexible forceps with opposing 2 - 3 mm cups on their distal end are manufactured with numerous configurations (i.e., cups can be smooth or serrated, standard or fenestrated, with, or without, a central needle; forceps may be multiple use or disposable, and some are designed to allow multiple samples to be taken before withdrawing the instrument; Fig. 2; Woods et al., 1999; Mansell and Willard, 2003; Padda et al., 2003). There is difference of opinion between endoscopists as to the best configuration for flexible endoscopic forceps. Fenestrated forceps may cause fewer crush artifacts and yield larger biopsy specimens than non-fenestrated models. Biopsy forceps with a central needle can help stabilize forceps on the mucosa and are useful for some endoscopists but may yield inferior tissue samples associated with puncture artifacts in the hands of other endoscopsists (Mansell and Willard, 2003). Others have shown that forceps cup size is what matters most by demonstrating that large capacity forceps (with, or without, a central spike) provided the highest quality of duodenal samples obtained from healthy dogs (Goutal-Landry et al., 2013). The use of forceps that can be passed through smaller diameter endoscopes has also been shown to yield excellent quality endoscopic specimens of the ileum, or in animals < 10 kg because the mucosa is relatively thin (Willard et al., 2001).

Both single- and multi-use forceps may be used to procure mucosal samples and they require proper maintenance. Of interest, single-use biopsy forceps may be used in multiple GI procedures as long as the forceps remain sharp and their function is well maintained. Additionally, disposable (single use) forceps are quite cost-effective (Bourguignon et al., 2003).

Careful and thorough manual cleaning of forceps with water and an enzymatic agent should be performed shortly after the completion of any GI endoscopic procedure. Biopsy forceps are particularly difficult to clean and require autoclaving (i.e., steam under pressure) for effective sterilization due to their complicated mechanical structure (Yoon et al., 2012).

Tissue samples should be as large and free of artifact as possible because the diagnostic quality of the endoscopic sample influences the ability of the pathologist to detect and define mucosal lesions. In general, six marginal or adequate samples should be collected for abnormalities to be detected in the feline gastric and duodenal mucosa, whereas six adequate or 10 - 15 marginal samples should be collected from the canine stomach and duodenum, depending on the lesion being sought (Table 4; Willard et al., 2008). To be considered adequate, a biopsy sample should contain the full thickness of the mucosa and be wide enough to have at least three to four intact and preferably contiguous villi. Specimens containing submucosa are preferred, but it is not always possible to obtain tissue at this level, especially when the mucosa is relatively thick (e.g., large vs. small dog; duodenum vs. ileum).

There are various techniques for sampling GI mucosa with endoscopic forceps which are reviewed in detail elsewhere (Woods et al., 1999; Mansell and Willard, 2003; Padda et al., 2003). Endoscopic biopsies should always be obtained. Even animals with mucosa that appears normal may have important histopathologic lesions. Gastric biopsies are usually easy to obtain, especially from rugal folds in the gastric body or from the fundus. Good samples from the antrum near the pylorus are more difficult, because the tissue is more dense and harder to tear off. The duodenum is typically the most difficult organ to obtain good tissue samples from

because of the difficulty in positioning the endoscopic forceps perpendicular (90°) to the mucosa. Larger dogs may also have a thicker duodenal mucosa, which makes it difficult to obtain full-thickness of the mucosa. Use of an endoscope that allows 2.8 mm forceps is crucial in larger dogs. Good quality duodenal biopsies occasionally leave behind an opaque base in the mucosal defect, indicating the procurement of full-thickness mucosa down to the muscularis mucosa (Fig. 3). Ileal biopsies can provide a diagnosis that is unavailable from duodenal biopsies (especially in cats; Scott et al., 2011) and/or contain histopathologic lesions that differ from duodenal biopsies (Casamian-Sorrosal et al., 2010; Procoli et al., 2013). It is typically easy to obtain high quality ileal biopsies because ileal mucosa is relatively thin, allowing for full thickness specimens with minimal effort (Fig. 4). Colonic mucosa is sampled in a similar fashion to duodenal mucosa, with endoscopic biopsies routinely obtained from the descending, transverse, and ascending colon. The colonic mucosa is also relatively thin, making it easy to obtain good quality tissue samples.

Disposable guarded cytology brushes are useful for obtaining cell specimens during endoscopic examination. Once the mucosal area to be sampled is identified, superficial cells are obtained, placed onto microscopic slides, and examined microscopically for evidence of inflammation, neoplasia, or infectious agents. In one comprehensive investigation, brush and touch cytologic specimens obtained by endoscopic examination of the stomach (n = 49), small intestine (n = 47), and colon (n = 18) in 44 dogs and 14 cats showed excellent correlation to histopathologic findings (Jergens et al., 1998). The sensitivity and specificity, respectively, of endoscopic cytologic specimens for the detection of abnormalities, was 100% and 92% for the stomach, 93% and 93% for the small intestine, and 88% and 88% for the colon. A similar

diagnosis was made for both cytologic and histopathologic specimens determined to be normal or to have lymphoplasmacytic inflammation, mixed inflammation, eosinophilic inflammation, and lymphoid malignancy involving the GI mucosa. Results from cytological samples often provide more rapid turnaround time than histopathologic interpretation of mucosal specimens.

Post-biopsy considerations to enhance correct histopathologic diagnosis

Endoscopic tissue specimens are small and fragile, subject to artifact from handling, mounting, and processing (including microtome sectioning of the paraffin wax-embedded tissue). Careful specimen handling, avoidance and recognition of routine (e.g. hematoxylin and eosin, H and E) tissue artifacts, and consideration of other tissue fixative options for immunohistochemistry and molecular testing, optimizes diagnostic interpretation (Fig. 5).

'After endoscopic biopsy collection, how can I maximize the diagnostic yield of my mucosal samples? What factors influence quality of the histopathologic interpretation?'

Biopsy specimen handling

Tissue specimens should be gently teased from the forceps with a needle and placed on lens paper, cucumber slice, or specially designed biopsy sponges. Commercial cassettes with pre-cut 'sponges' can be used for the submission of endoscopic biopsy specimens. Multiple biopsy specimens can be arranged on a sponge and the sponge and the closed cassette is then immersed in 10% neutral buffered formalin and submitted to the laboratory.

Cucumber slices can be substituted for plastic sponges and are an excellent medium for the submission of endoscopic biopsy specimens (Table 5; Swan and Davis, 1970; Murray et al.,

2007). Biopsy samples are arranged in parallel rows on the thin slices of cucumber (preserved in alcohol), which are then deposited in formalin and submitted for routine processing (Fig. 6). At the laboratory, the cucumber cassettes are removed from the formalin and the cucumber sections are reoriented 90° on their side in the cassette (e.g., perpendicular to the cassette surface to optimize tissue orientation after sectioning). The tissues are then embedded in paraffin wax. The specimens do not have to be removed from the cucumber slices prior to embedding because the microtome can readily cut through the vegetable material. This technique minimizes specimen handling at the laboratory and consistently yields well-oriented tissues of high diagnostic quality.

Attempts to reorient specimens on biopsy sponges or cucumber slices prior to formalin fixation should be avoided. Specimens should not be allowed to dry out on cucumber or sponge surfaces. Overly dried samples may adhere tightly to the sponge or cucumber and be damaged when removed by the pathology service. Samples from different sites (e.g., stomach, duodenum, and colon) should be placed in separate containers and appropriately labeled. The endoscopist should record the number of specimens obtained from each site, relevant endoscopic observations, and salient historical and clinical data on the histopathology form. An example of endoscopic report forms that may be downloaded for use can be found online.[1]

Tissue artifacts

Various artifacts hinder accurate interpretation of endoscopic biopsy specimens. When placed in formalin, the mucosa of GI tissues has a tendency to roll over the submucosa, making precise orientation prior to routine processing difficult. Multiple samples are embedded in the same paraffin wax block, and 3-4 micron sections are shaved from the block until the section

[1] See: http://www.wsava.org/educational/gastrointestinal-standardization-project (Accessed 13 April 2016)

obtained represents the largest specimen of each piece of tissue. Many of the sections may have oblique orientation, and if the mucosa has significant rollover artifact, the surface of some specimens may be the only tissue available for microscopic examination. Hence, some small intestinal biopsy sections may consist of villi only (Fig. 7A). In these instances, it is not possible to evaluate the subvillus lamina propria. Other sections may be devoid of surface epithelia, creating the false impression of mucosal ulceration. Where villi are cut tangentially, the impression of villus stunting might be obtained by the untrained observer. Irregular or apparently multilayered epithelium at the 'tip' of such villi indicates that they have not been cut in perpendicular orientation.

Pinch or stretch artifacts created at the margins of biopsy specimens (Fig. 7B) are evidenced by the 'telescoping' of mucosal glands, the expression of mucosal glands from the underlying lamina propria into the area of the lumen, and 'streaming' of nuclear chromatin. To some extent these changes are unavoidable. Good biopsy technique (especially avoiding rapid closure of the biopsy forceps during tissue procurement) and gentle handling of specimens after biopsy can minimize these artifacts. Streaming nuclear chromatin can be particularly problematic in tissues with fragile cells (e.g., lymphoma). If lymphoma is suspected, the endoscopist should be especially gentle with the specimen prior to fixation, and additional biopsy samples should be obtained, to maximize the likelihood of a diagnostic specimen. Additionally, exfoliative cytologic specimens obtained by biopsy 'imprints' (rather than smears) onto glass slides often yield excellent quality specimens for diagnostic review (Fig. 8). Fresh biopsy specimens should also be obtained for culture/sensitivity testing of invasive *E. coli* in breeds at risk for developing granulomatous colitis (e.g., Boxers, French Bulldog).

Tissue fixation, immunohistochemistry and molecular testing

Fixation in 10% neutral buffered formalin is adequate for routine histologic examination. Glutaraldehyde fixation is optimal for electron microscopy. Immunohistochemical labeling of certain cell-associated antigens is increasingly available to diagnostic histopathologists (e.g., phenotypic classification of alimentary lymphoma; Waly et al., 2005). Although standard antibody panels may be applied to fixed tissue taken from the same wax block used for routine H and E sections, the use of more specialized immunohistochemistry may require tissue samples snap frozen in liquid nitrogen or preserved in fixatives other than formalin (e.g., alcohol). A discussion with the pathologist before undertaking endoscopy can ensure that appropriate specimens are collected. Tissues preserved by snap freezing or placed in RNAlater (Qiagen) may be used for extraction of nucleic acids (e.g., RNA or DNA) utilized in molecular testing, such as PCR (performed on DNA extracted from mucosal tissues), or fluorescence in situ hybridization (FISH, performed on formalin-fixed serial tissue sections that have undergone de-paraffinization prior to probe hybridization) techniques (Fig. 9).

Standard H and E staining provides excellent tissue detail for routine microscopic examination. Special staining may highlight certain infectious agents, but is no substitute for culture or PCR-based detection and speciation of organisms. A variety of silver impregnation methods and Giemsa staining have been employed to identify *Helicobacter* spp. organisms in gastric biopsy specimens. Fungi can be identified with periodic acid–Schiff reagents (PAS) or silver techniques (e.g., Gomori's methanamine silver stain). Bacteria can be assessed in PAS or Gram-stained sections of tissue. Stains for collagen fibers may aid in evaluating fibrosis, and

PAS stain highlights colonic mucus and macrophages in granulomatous colitis of Boxer dogs (Fig. 10).

Immunohistochemical or immunofluorescence methods have been applied to endoscopic biopsy tissues and are now becoming routinely available. Immunohistochemistry has become especially useful for diagnosis of feline alimentary lymphoma, which represents one of the greatest diagnostic challenges for pathologists because of suspected transition between lymphoplasmacytic inflammation and lymphoma (Bridgeford et al., 2008). Labeling of serial sections with antisera specific for CD3 (a pan-T-cell marker) and CD79a (a pan-B-cell marker) can help the pathologist determine the clonality of an infiltrate. In the cat, this basic immunolabeling also helps to distinguish well differentiated (small cell = lymphocytic = T-cell) from lymphoblastic (large cell = lymphoblastic = B-cell) alimentary lymphoma (Fig. 11).

A range of antisera has been applied to studies of canine and feline IBD to phenotype and enumerate infiltrating populations of T lymphocytes (CD3+, CD4+ and CD8+, antisera specific for the canine T-cell receptors of α-β or γ-δ chain composition), B lymphocytes (CD79a), plasma cells (IgG, IgM and IgA), mast cells (IgE) and antigen presenting cells (MAC387, MHC class II, CD1, CD11c), as well as tight-junction proteins (Kathrani et al., 2011; Rossi et al., 2014). Most of these specialized tests for cell surface immune protein expression can be performed retrospectively on formalin-fixed tissue sections.

Controversies in histopathologic interpretation of endoscopic biopsies

Histopathologic interpretation may vary according to the quality of tissue specimens submitted (related to operator experience and processing artifacts) and inconsistent histopathologic criteria for defining GI inflammation in dogs and cats.

'Does the quality of endoscopic specimens submitted to the laboratory affect histopathologic interpretation?'

Yes, there is an association between quality of the endoscopic sample and histopathologic interpretation. Van der Gaag and Happe examined 340 tissue specimens obtained by endoscopic forceps from 151 dogs and reported that 77 (23%) were unsuitable for pathologic examination (van der Gaag and Happe, 1990). In a more contemporary study (Willard et al., 2001), the quality of endoscopic specimens collected by different endoscopists and submitted to two different laboratories were evaluated. One set of tissues was submitted by an experienced endoscopist or by individuals trained by that clinician in proper biopsy collection and submission to the laboratory (laboratory 1). In these instances, the endoscopic specimens were believed to be of good quality and were carefully oriented on a plastic sponge prior to formalin fixation. The second laboratory evaluated endoscopic specimens obtained by multiple, less experienced endoscopists comprising a multi-practice environment (laboratory 2). These tissue specimens were submitted floating free in various-sized containers of formalin. Biopsy specimens from each laboratory were scored as clearly adequate, clearly inadequate, or of questionable adequacy for histopathologic diagnosis. Results indicated that laboratory 1 samples were superior to laboratory 2 samples, with significantly more laboratory 1 tissues likely to be scored as clearly adequate in depth and size. An important finding of this study was that overall tissue score was associated with the number of individual tissues per slide – more tissue specimens placed on a

445 slide resulted in a greater percentage of clearly adequate tissues obtained. These findings led to
446 the recommendation that at least eight individual tissue specimens should be submitted when
447 performing endoscopic biopsy of the duodenum in dogs and cats.
448
449 Other factors may affect endoscopic biopsy quality, including operator experience for the
450 detection of mucosal lesions and the effect of tissue processing on histopathologic assessment
451 (Willard et al., 2001). Slovak demonstrated that use of descriptive terms accompanied by
452 pictures of representative mucosal abnormalities significantly improved the diagnostic accuracy
453 of novice endoscopists to almost that of experienced endoscopists (i.e., advanced clinical training
454 and active operator participation in GI endoscopy over the preceding 24 months) (Slovak et al.,
455 2014). Finally, the use of a different pictorial template for grading intestinal lesions failed to
456 improve the consistency of diagnostic interpretation between pathologists, because of differences
457 in slide processing (Willard et al., 2008).
458
459 *'Are histopathologic guidelines for endoscopic biopsies presently in place?'*
460 Yes, but uniform grading criteria for defining GI inflammation in endoscopic specimens
461 remain controversial (Washabau et al., 2010; Simpson and Jergens, 2011). Over the past two
462 decades, numerous grading schemes for characterizing the nature and severity GI inflammation
463 have been designed (Jergens et al., 1992; Wilcock, 1992; German et al., 2001; Waly et al., 2004;
464 Allenspach et al., 2007; Garcia-Sancho et al., 2007). Most of these model systems emphasize the
465 type and degree of lamina propria cellular infiltrate that is subjectively characterized as normal,
466 mild, moderate, or severe. It should be noted that different types of cellular infiltrates (e.g.,
467 lymphoplasmacytic, eosinophilic, and granulomatous) are recognized and that these populations

overlap and occur in various combinations with different GI diseases. The emphasis on mucosal cellularity has meant that abnormalities in mucosal architecture have been overlooked, even though they may correlate with inflammatory markers and clinical severity (Wiinberg et al., 2005; Janeczko et al., 2008). Moreover, mean cell populations (e.g., CD3+ T cells) do not differ in IBD dogs at diagnosis vs. when in clinical remission (Schreiner et al., 2008), and cats with and without signs of intestinal disease may have similar numbers of lymphocytes and plasma cells (Waly et al., 2004).

These findings and the observations that GI histopathologic interpretation varies widely between pathologists (Willard et al., 2002) have led to new standardized criteria for defining gut inflammation (Day et al., 2008). The World Small Animal Veterinary Association (WSAVA) Standardization scheme uses eight morphologic and inflammatory features to assign an inflammatory score of normal, mild, moderate, or severe/marked with a final diagnosis that describes the predominant abnormalities. A limitation of the WSAVA scheme is that it does not account for goblet cells, which are considered important in colonic disease (Roth et al., 1990b; Mansfield et al., 2009). A simplified pathologic model, using the WSAVA criteria that showed the most consistency in interpretation and including enumeration of goblet cells, has been recently described (Jergens et al., 2014).

Correlation of Histopathologic findings to clinical and endoscopic indices

An ever-increasing number of clinicians perform endoscopic mucosal biopsy in dogs and cats with chronic or recurrent GI signs. There is strong expectation that the histopathologic

features contained in tissue samples will confirm a diagnosis and guide treatment decisions in these instances (Mansell and Willard, 2003).

'What is the association, if any, between histopathologic findings and clinical and endoscopic indices?'

A variety of factors including host genetics, mucosal immunity, environmental factors (i.e., diet, intestinal microbiota), and defects in GI function (motility) may variably impact the severity of clinical signs and histopathologic inflammation in dogs and cats with CE (Allenspach, 2011; Simpson and Jergens, 2011). With regard to the value of endoscopic specimens, inconsistency between pathologists (Willard et al., 2002), questionable quality of tissues submitted to the laboratory (Willard et al., 2001), and controversy regarding which grading scheme to utilize (Day et al., 2008) are all factors that have made it difficult to accurately correlate histopathologic findings to clinical disease activity. Different trials at different institutions have attempted to correlate histopathologic changes in endoscopic specimens to disease severity at diagnosis or in response to treatment. For dogs with CE (predominantly IBD), the collective results indicated that there was no significant association between histopathologic findings and clinical signs, serum biomarkers, or responses to different treatments (Craven et al., 2004; Allenspach et al., 2007; Garcia-Sancho et al., 2007; McCann et al., 2007; Schreiner et al., 2008). In one study, Jergens (Jergens et al., 2003b) demonstrated that the canine IBD activity index had good correlation to histologic and biomarker scores in canine IBD. Another study modified this existing index (e.g., canine chronic enteropathy clinical activity index) and demonstrated that high clinical disease activity, but not histopathologic lesion score, was associated with negative long-term outcome (Allenspach et al., 2007).

Detection of the extent and severity of mucosal disease is aided by the use of endoscopic indices in humans with IBD (Daperno et al., 2004; Osada et al., 2010). There are relatively few reports in dogs where endoscopic lesions were associated with clinical severity and histopathologic lesions (Roth et al., 1990a; Jergens et al., 1992; Jergens et al., 2003a; Allenspach et al., 2007; Garcia-Sancho et al., 2007; Schreiner et al., 2008). Separate studies in dogs with small intestinal IBD have yielded conflicting results about the utility of endoscopic scoring as a measure of disease activity (Allenspach et al., 2007; Garcia-Sancho et al., 2007). These discordant results might be partially explained by differences in operator experience in detecting mucosal lesions of inflammation. The use of a simple endoscopic activity score based on qualitative criteria (e.g., friability, granularity, erosions, and lymphatic dilatation) has been recently validated in dogs with histopathologic IBD (Table 6; Slovak et al., 2015).

Despite limitations in endoscopic biopsy quality and histologic grading, advances are being made. Willard (Willard et al., 2008) demonstrated that histopathologic lesions of intestinal lymphangiectasia were correctly identified by most pathologists in dogs with hypoproteinemia. The obvious utility of collecting ileal biopsies to aid in the differentiation of feline lymphoma from severe enteropathy (Evans et al., 2006; Kleinschmidt et al., 2010; Scott et al., 2011), and the recognition that ileal and duodenal mucosa differ in the character/severity of inflammation, has improved diagnostic accuracy (Casamian-Sorrosal et al., 2010; Procoli et al., 2013). Finally, molecular testing performed on endoscopic biopsies is gaining popularity in clinical practice for the evaluation of specific bacterial pathogens (Hostutler et al., 2004; Janeczko et al., 2008;

Jergens et al., 2009), microbial abundance (Xenoulis et al., 2008; Suchodolski et al., 2010; Suchodolski et al., 2012), and host gene expression profiles (Wilke et al., 2012).

Conclusions

Endoscopic biopsy has a primary role in morphological investigations of the upper and lower GI tract in dogs and cats. The value of endoscopic biopsy is influenced by the following caveats: (1) endoscopic biopsy is not indicated in all animals with GI disease, especially those in which appropriate therapeutic trials (e.g., deworming, dietary modification, antimicrobial trial for antimicrobial-responsive diarrheas) have not been performed; (2) mucosal biopsies should always be collected when performing GI endoscopy; biopsy guidelines are now established and recent studies indicate that operator experience influences both endoscopic mucosal assessment and the quality of the endoscopic biopsy specimen collected; (3) adequate numbers of high quality specimens should be submitted to enhance diagnostic accuracy; (4) ileal biopsies should always be obtained, even 'blind' biopsies through the ileocolic valve are required to do so; (5) endoscopic specimen quality should be optimized by careful tissue removal from forceps, proper biopsy orientation, and submission to a laboratory skilled in endoscopic histopathologic interpretation; and (6) histopathologic guidelines for biopsy interpretation remain fluid, since standardized criteria for mucosal inflammation have not been embraced by all pathologists. The WSAVA histopathologic score is often utilized and includes key morphologic and inflammatory features (with the exception of goblet cells) relevant to GI inflammation in dogs and cats.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with people or organizations that could inappropriately influence or bias the content of the paper.

References

Allenspach, K., 2011. Clinical immunology and immunopathology of the canine and feline intestine. The Veterinary Clinics of North America. Small Animal Practice 41, 345-360.

Allenspach, K., Wieland, B., Grone, A., Gaschen, F., 2007. Chronic enteropathies in dogs: evaluation of risk factors for negative outcome. Journal of Veterinary Internal Medicine 21, 700-708.

Baez, J.L., Hendrick, M.J., Walker, L.M., Washabau, R.J., 1999. Radiographic, ultrasonographic, and endoscopic findings in cats with inflammatory bowel disease of the stomach and small intestine: 33 cases (1990-1997). Journal of the American Veterinary Medical Association 215, 349-354.

Baron, J.H., Connell, A.M., Lennard-Jones, J.E., 1964. Variation between observers in describing mucosal appearances in proctocolitis. British Medical Journal 1, 89-92.

Bourguignon, C., Destrumelle, A.S., Koch, S., Grumblat, A., Carayon, P., Chopard, C., Woronoff-Lemsi, M.C., 2003. Disposable versus reusable biopsy forceps in GI endoscopy: A cost-minimization analysis. Gastrointestinal Endoscopy 58, 226-229.

Bridgeford, E.C., Marini, R.P., Feng, Y., Parry, N.M., Rickman, B., Fox, J.G., 2008. Gastric Helicobacter species as a cause of feline gastric lymphoma: A viable hypothesis. Veterinary Immunology and Immunopathology 123, 106-113.

Casamian-Sorrosal, D., Willard, M.D., Murray, J.K., Hall, E.J., Taylor, S.S., Day, M.J., 2010. Comparison of histopathologic findings in biopsies from the duodenum and ileum of dogs with enteropathy. Journal of Veterinary Internal Medicine 24, 80-83.

Craven, M., Simpson, J.W., Ridyard, A.E., Chandler, M.L., 2004. Canine inflammatory bowel disease: retrospective analysis of diagnosis and outcome in 80 cases (1995-2002). Journal of Small Animal Practice 45, 336-342.

Daperno, M., D'Haens, G., Van Assche, G., Baert, F., Bulois, P., Maunoury, V., Sostegni, R., Rocca, R., Pera, A., Gevers, A., et al., 2004. Development and validation of a new, simplified endoscopic activity score for Crohn's disease: the SES-CD. Gastrointestinal Endoscopy 60, 505-512.

Day, M.J., Bilzer, T., Mansell, J., Wilcock, B., Hall, E.J., Jergens, A., Minami, T., Willard, M., Washabau, R., 2008. 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. Journal of Compomparative Pathology 138, S1-43.

Evans, S.E., Bonczynski, J.J., Broussard, J.D., Han, E., Baer, K.E., 2006. Comparison of endoscopic and full-thickness biopsy specimens for diagnosis of inflammatory bowel disease and alimentary tract lymphoma in cats. Journal of the American Veterinary Medical Association 229, 1447-1450.

Garcia-Sancho, M., Rodriguez-Franco, F., Sainz, A., Mancho, C., Rodriguez, A., 2007. Evaluation of clinical, macroscopic, and histopathologic response to treatment in nonhypoproteinemic dogs with lymphocytic-plasmacytic enteritis. Journal of Veterinary Internal Medicine 21, 11-17.

Garcia-Sancho, M., Sainz, A., Villaescusa, A., Rodriguez, A., Rodriguez-Franco, F., 2011. White spots on the mucosal surface of the duodenum in dogs with lymphocytic plasmacytic enteritis. Journal of Veterinary Science 12, 165-169.

German, A.J., Hall, E.J., Day, M.J., 2001. Immune cell populations within the duodenal mucosa of dogs with enteropathies. Journal of Veterinary Internal Medicine 15, 14-25.

Gieger, T., 2011. Alimentary lymphoma in cats and dogs. The Veterinary Clinics of North America. Small Animal Practice 41, 419-432.

Goutal-Landry, C.M., Mansell, J., Ryan, K.A., Gaschen, F.P., 2013. Effect of endoscopic forceps on quality of duodenal mucosal biopsy in healthy dogs. Journal of Veterinary Internal Medicine 27, 456-461.

Heilmann, R.M., Grellet, A., Allenspach, K., Lecoindre, P., Day, M.J., Priestnall, S.L., Toresson, L., Procoli, F., Grutzner, N., Suchodolski, J.S., et al., 2014. Association between fecal S100A12 concentration and histologic, endoscopic, and clinical disease severity in dogs with idiopathic inflammatory bowel disease. Veterinary Immunology and Immunopathology 158, 156-166.

Heilmann, R.M., Jergens, A.E., Ackermann, M.R., Barr, J.W., Suchodolski, J.S., Steiner, J.M., 2012. Serum calprotectin concentrations in dogs with idiopathic inflammatory bowel disease. American Journal of Veterinary Research 73, 1900-1907.

Hostutler, R.A., Luria, B.J., Johnson, S.E., Weisbrode, S.E., Sherding, R.G., Jaeger, J.Q., Guilford, W.G., 2004. Antibiotic-responsive histiocytic ulcerative colitis in 9 dogs. Journal of Veterinary Internal Medicine 18, 499-504.

Janeczko, S., Atwater, D., Bogel, E., Greiter-Wilke, A., Gerold, A., Baumgart, M., Bender, H., McDonough, P.L., McDonough, S.P., Goldstein, R.E., et al., 2008. The relationship of mucosal bacteria to duodenal histopathology, cytokine mRNA, and clinical disease activity in cats with inflammatory bowel disease. Veterinary Microbiology 128, 178-193.

646 Jergens, A.E., 2012. Feline idiopathic inflammatory bowel disease: what we know and what
647 remains to be unraveled. Journal of Feline Medicine and Surgery 14, 445-458.
648
649 Jergens, A.E., Andreasen, C.B., Hagemoser, W.A., Ridgway, J., Campbell, K.L., 1998.
650 Cytologic examination of exfoliative specimens obtained during endoscopy for diagnosis
651 of gastrointestinal tract disease in dogs and cats. Journal of the American Veterinary
652 Medical Association 213, 1755-1759.
653
654 Jergens, A.E., Evans, R.B., Ackermann, M., Hostetter, J., Willard, M., Mansell, J., Bilzer, T.,
655 Wilcock, B., Washabau, R., Hall, E.J., et al., 2014. Design of a simplified histopathologic
656 model for gastrointestinal inflammation in dogs. Veterinary Pathology 51, 946-950.
657
658 Jergens, A.E., Moore, F.M., Haynes, J.S., Miles, K.G., 1992. Idiopathic inflammatory bowel
659 disease in dogs and cats: 84 cases (1987-1990). Journal of the American Veterinary
660 Medical Association 201, 1603-1608.
661
662 Jergens, A.E., Pressel, M., Crandell, J., Morrison, J.A., Sorden, S.D., Haynes, J., Craven, M.,
663 Baumgart, M., Simpson, K.W., 2009. Fluorescence in situ hybridization confirms
664 clearance of visible Helicobacter spp. associated with gastritis in dogs and cats. Journal
665 of Veterinary Internal Medicine 23, 16-23.
666
667 Jergens, A.E., Schreiner, C.A., Frank, D.E., Niyo, Y., Ahrens, F.E., Eckersall, P.D., Benson, T.J.,
668 Evans, R., 2003b. A scoring index for disease activity in canine inflammatory bowel
669 disease. Journal of Veterinary Internal Medicine 17, 291-297.
670
671 Jergens AE, Willard MD, Day MJ., 2011 Endoscopic biopsy specimen collection and
672 histopathologic considerations. In: Tams TR and Rawlings CA (eds.), Small Animal
673 Endoscopy, Elsevier, USA, pp. 293-309.
674
675 Kathrani, A., Schmitz, S., Priestnall, S.L., Smith, K.C., Werling, D., Garden, O.A., Allenspach,
676 K., 2011. CD11c + cells are significantly decreased in the duodenum, ileum and colon of
677 dogs with inflammatory bowel disease. Journal of Comparative Pathology 145, 359-366.
678
679 Kleinschmidt, S., Harder, J., Nolte, I., Marsilio, S., Hewicker-Trautwein, M., 2010. Chronic
680 inflammatory and non-inflammatory diseases of the gastrointestinal tract in cats:
681 diagnostic advantages of full-thickness intestinal and extraintestinal biopsies. Journal of
682 Feline Medicine and Surgery 12, 97-103.
683
684 Larson, R.N., Ginn, J.A., Bell, C.M., Davis, M.J., Foy, D.S., 2012. Duodenal endoscopic
685 findings and histopathologic confirmation of intestinal lymphangiectasia in dogs. Journal
686 of Veterinary Internal Medicine 26, 1087-1092.
687
688 Lingard, A.E., Briscoe, K., Beatty, J.A., Moore, A.S., Crowley, A.M., Krockenberger, M.,
689 Churcher, R.K., Canfield, P.J., Barrs, V.R., 2009. Low-grade alimentary lymphoma:
690 clinicopathological findings and response to treatment in 17 cases. Journal of Feline
691 Medicine and Surgery 11, 692-700.

Mansell, J., Willard, M.D., 2003. Biopsy of the gastrointestinal tract. Veterinary Clinics of North America, Small Animal Practice 33, 1099-1116.

Mansfield, C.S., James, F.E., Craven, M., Davies, D.R., O'Hara, A.J., Nicholls, P.K., Dogan, B., MacDonough, S.P., Simpson, K.W., 2009. Remission of histiocytic ulcerative colitis in Boxer dogs correlates with eradication of invasive intramucosal Escherichia coli. Journal of Veterinary Internal Medicine 23, 964-969.

McCann, T.M., Ridyard, A.E., Else, R.W., Simpson, J.W., 2007. Evaluation of disease activity markers in dogs with idiopathic inflammatory bowel disease. Journal of Small Animal Practice 48, 620-625.

Murray, C.E., Cooper, L., Handa, K.K., MacLeod, T., MacKenzie, K., 2007. A technique for the orientation of endoscopically resected laryngeal lesions. Clinical otolaryngology : official journal of ENT-UK ; official journal of Netherlands Society for Oto-Rhino-Laryngology and Cervico-Facial Surgery 32, 201-203.

Osada, T., Ohkusa, T., Yokoyama, T., Shibuya, T., Sakamoto, N., Beppu, K., Nagahara, A., Otaka, M., Ogihara, T., Watanabe, S., 2010. Comparison of several activity indices for the evaluation of endoscopic activity in UC: inter- and intraobserver consistency. Inflammatory Bowel Disease 16, 192-197.

Padda, S., Shah, I., Ramirez, F.C., 2003. Adequacy of mucosal sampling with the 'two-bite' forceps technique: a prospective, randomized, blinded study. Gastrointestinal Endoscopy 57, 170-173.

Procoli, F., Motskula, P.F., Keyte, S.V., Priestnall, S., Allenspach, K., 2013. Comparison of histopathologic findings in duodenal and ileal endoscopic biopsies in dogs with chronic small intestinal enteropathies. Journal of Veterinary Internal Medicine 27, 268-274.

Rath, T., Tontini, G.E., Neurath, M.F., Neumann, H., 2015. From the surface to the single cell: Novel endoscopic approaches in inflammatory bowel disease. World Journal of Gastroenterology 21, 11260-11272.

Richter, K.P., Cleveland, M.B., 1989. Comparison of an orally administered gastrointestinal lavage solution with traditional enema administration as preparation for colonoscopy in dogs. Journal of the American Veterinary Medical Association 195, 1727-1731.

Rossi, G., Pengo, G., Caldin, M., Piccionello, A.P., Steiner, J.M., Cohen, N.D., Jergens, A.E., Suchodolski, J.S., 2014. Comparison of microbiological, histological, and immunomodulatory parameters in response to treatment with either combination therapy with prednisone and metronidazole or probiotic VSL#3 Strains in dogs with idiopathic inflammatory bowel disease. PLoS ONE 9, e94699.

737 Roth, L., Leib, M.S., Davenport, D.J., Monroe, W.E., 1990a. Comparisons between endoscopic
738 and histologic evaluation of the gastrointestinal tract in dogs and cats: 75 cases (1984-
739 1987). Journal of the American Veterinary Medical Association 196, 635-638.
740
741 Roth, L., Walton, A.M., Leib, M.S., Burrows, C.F., 1990b. A grading system for lymphocytic
742 plasmacytic colitis in dogs. Journal of Veterinary Diagnostic Investigation 2, 257-262.
743
744 Schreiner, N.M.S., Gaschen, F., Grone, A., Sauter, S.N., Allenspach, K., 2008. Clinical signs,
745 histology, and CD3-positive cells before and after treatment of dogs with chronic
746 enteropathies. Journal of Veterinary Internal Medicine 22, 1079-1083.
747
748 Scott, K.D., Zoran, D.L., Mansell, J., Norby, B., Willard, M.D., 2011. Utility of endoscopic
749 biopsies of the duodenum and ileum for diagnosis of inflammatory bowel disease and
750 small cell lymphoma in cats. Journal of Veterinary Internal Medicine 25, 1253-1257.
751
752 Sharman, M.J., Bacci, B., Whittem, T., Mansfield, C.S., 2013. In vivo confocal endomicroscopy
753 of small intestinal mucosal morphology in dogs. Journal of Veterinary Internal Medicine
754 27, 1372-1378.
755
756 Sharman, M.J., Bacci, B., Whittem, T., Mansfield, C.S., 2014. In vivo histologically equivalent
757 evaluation of gastric mucosal topologic morphology in dogs by using confocal
758 endomicroscopy. Journal of Veterinary Internal Medicine 28, 799-808.
759
760 Simpson, J.W., Else, R.W., 1987. Diagnostic value of tissue biopsy in gastrointestinal and liver
761 disease. Veterinary Record 120, 230-233.
762
763 Simpson, K.W., Jergens, A.E., 2011. Pitfalls and progress in the diagnosis and management of
764 canine inflammatory bowel disease. Veterinary Clinics of North America, Small Animal
765 Practice 41, 381-398.
766
767 Slovak, J.E., Wang, C., Morrison, J.A., Deitz, K.L., LeVine, D.N., Otoni, C., King, R.R., Gerber,
768 L.E., Hanson, K.R., Lundberg, A.P., et al., 2014. Endoscopic assessment of the
769 duodenum in dogs with inflammatory bowel disease. Journal of Veterinary Internal
770 Medicine 28, 1442-1446.
771
772 Slovak, J.E., Wang, C., Sun, Y., Otoni, C., Morrison, J., Deitz, K., LeVine, D., Jergens, A.E.,
773 2015. Development and validation of an endoscopic activity score for canine
774 inflammatory bowel disease. The Veterinary Journal 203, 290-295.
775
776 Suchodolski, J.S., Dowd, S.E., Wilke, V., Steiner, J.M., Jergens, A.E., 2012. 16S rRNA gene
777 pyrosequencing reveals bacterial dysbiosis in the duodenum of dogs with idiopathic
778 inflammatory bowel disease. PLoS ONE 7, e39333.
779
780 Suchodolski, J.S., Xenoulis, P.G., Paddock, C.G., Steiner, J.M., Jergens, A.E., 2010. Molecular
781 analysis of the bacterial microbiota in duodenal biopsies from dogs with idiopathic
782 inflammatory bowel disease. Veterinary Microbiology 142, 394-400.

Sutherland-Smith, J., Penninck, D.G., Keating, J.H., Webster, C.R., 2007. Ultrasonographic intestinal hyperechoic mucosal striations in dogs are associated with lacteal dilation. Veterinary Radiology and Ultrasound 48, 51-57.

Swan, R.W., Davis, H.J., 1970. The biopsy-cucumber unit. A method to improve tissue orientation. Obstetrics and Gynecology 36, 803-805.

van der Gaag, I., Happe, R.P., 1990. The histological appearance of peroral small intestinal biopsies in clinically healthy dogs and dogs with chronic diarrhea. Zentralblatt fur Veterinarmedizin A 37, 401-416.

Waly, N.E., Gruffydd-Jones, T.J., Stokes, C.R., Day, M.J., 2005. Immunohistochemical diagnosis of alimentary lymphomas and severe intestinal inflammation in cats. Journal of Comparative Pathology 133, 253-260.

Waly, N.E., Stokes, C.R., Gruffydd-Jones, T.J., Day, M.J., 2004. Immune cell populations in the duodenal mucosa of cats with inflammatory bowel disease. Journal of Veterinary Internal Medicine 18, 816-825.

Washabau, R.J., Day, M.J., Willard, M.D., Hall, E.J., Jergens, A.E., Mansell, J., Minami, T., Bilzer, T.W., 2010. Endoscopic, biopsy, and histopathologic guidelines for the evaluation of gastrointestinal inflammation in companion animals. Journal of Veterinary Internal Medicine 24, 10-26.

Webb, C.B., 2008. Feline laparoscopy for gastrointestinal disease. Topics in Companion Animal Medicine 23, 193-199.

Wiinberg, B., Spohr, A., Dietz, H.H., Egelund, T., Greiter-Wilke, A., McDonough, S.P., Olsen, J., Priestnall, S., Chang, Y.F., Simpson, K.W., 2005. Quantitative analysis of inflammatory and immune responses in dogs with gastritis and their relationship to Helicobacter spp. infection. Journal of Veterinary Internal Medicine 19, 4-14.

Wilcock, B., 1992. Endoscopic biopsy interpretation in canine or feline enterocolitis. Seminars in Veterinary Medicine and Surgery (Small Animal) 7, 162-171.

Wilke, V.L., Nettleton, D., Wymore, M.J., Gallup, J.M., Demirkale, C.Y., Ackermann, M.R., Tuggle, C.K., Ramer-Tait, A.E., Wannemuehler, M.J., Jergens, A.E., 2012. Gene expression in intestinal mucosal biopsy specimens obtained from dogs with chronic enteropathy. American Journal of Veterinary Research 73, 1219-1229.

Willard, M.D., 2001. Colonoscopy, proctoscopy, and ileoscopy. Veterinary Clinics of North America, Small Animal Practice 31, 657-669, viii.

Willard, M.D., Jergens, A.E., Duncan, R.B., Leib, M.S., McCracken, M.D., DeNovo, R.C., Helman, R.G., Slater, M.R., Harbison, J.L., 2002. Interobserver variation among

histopathologic evaluations of intestinal tissues from dogs and cats. Journal of the American Veterinary Medical Association 220, 1177-1182.

Willard, M.D., Lovering, S.L., Cohen, N.D., Weeks, B.R., 2001. Quality of tissue specimens obtained endoscopically from the duodenum of dogs and cats. Journal of the American Veterinary Medical Association 219, 474-479.

Willard, M.D., Mansell, J., Fosgate, G.T., Gualtieri, M., Olivero, D., Lecoindre, P., Twedt, D.C., Collett, M.G., Day, M.J., Hall, E.J., et al., 2008. Effect of sample quality on the sensitivity of endoscopic biopsy for detecting gastric and duodenal lesions in dogs and cats. Journal of Veterinary Internal Medicine 22, 1084-1089.

Woods, K.L., Anand, B.S., Cole, R.A., Osato, M.S., Genta, R.M., Malaty, H., Gurer, I.E., Rossi, D.D., 1999. Influence of endoscopic biopsy forceps characteristics on tissue specimens: results of a prospective randomized study. Gastrointestinal Endoscopy 49, 177-183.

Xenoulis, P.G., Palculict, B., Allenspach, K., Steiner, J.M., House, A.M.v., Suchodolski, J.S., 2008. Molecular-phylogenetic characterization of microbial communities imbalances in the small intestine of dogs with inflammatory bowel disease. FEMS Microbiology Ecology 66, 579-589.

Yoon, J.H., Yoon, B.C., Lee, H.L., Lee, J.K., Kim, Y.T., Lee, D.H., Choi, I.J., Lee, D.H., Kim, D.H., 2012. Comparison of sterilization of reusable endoscopic biopsy forceps by autoclaving and ethylene oxide gas. Digestive Diseases and Science 57, 405-412.

Zoran, D.L., 2001. Gastroduodenoscopy in the dog and cat. Veterinary Clinics of North America, Small Animal Practice 31, 631-656.

858

859 **Figure legends**

860

861 Fig. 1. Representative still images used in the development of an endoscopic activity score:
862 A. normal stomach; B. erosions stomach; C. friability stomach; D. granularity stomach; E.
863 normal duodenum; F. erosions duodenum; G. friability duodenum; H. granularity duodenum; I.
864 lymphatic dilatation duodenum; J. normal colon; K. erosions colon; L. friability colon; M.
865 granularity colon; N. mass colon. All images are of canine GI mucosa.

866

867 Fig. 2. Photographs of the different types of available disposable forceps. #1: Alligator large
868 capacity with spike, #2: Alligator large capacity, #3: Alligator standard, #4: Alligator standard
869 with spike, #5: Standard oval, #6: Alligator pediatric (Goutal-Landry et al., 2013).

870

871 Fig. 3. Endoscopic biopsy of the duodenum showing a linear strip of mucosa that has been
872 removed. Note the appearance of the opaque *muscularis mucosa*, indicative of removal of an
873 excellent-quality mucosal specimen. Courtesy of MD Willard.

874

875 Fig. 4. 'Blind' biopsy technique of the canine ileum showing passage of the pinch forceps
876 through the ileocolic sphincter. Courtesy of MD Willard.

877

878 Fig. 5. Small intestinal biopsy specimen procured with pinch forceps from a healthy dog. Note
879 the excellent quality of this specimen, as evidenced by numerous intact villi, perpendicular
880 orientation of crypts to surface epithelium, and inclusion of deeper lamina propria tissue
881 (hematoxylin and eosin stain).

Fig. 6. Several duodenal biopsy specimens may be placed on cucumber slices before tissue processing. This minimizes specimen handling at the pathology laboratory.

Fig. 7. Poor-quality small intestinal biopsy specimen. (A) Note that tissues consist of villus tips only, without underlying subvillus *lamina propria* and associated structures. This type of tissue artifact may be caused by poor biopsy technique or a specimen rolling over during fixation. (B) Significant squeeze artifact at the base of the tissue specimen *(circle).* Artifacts of this type are sometimes difficult to avoid, even with good biopsy technique (both images are hematoxylin and eosin stain).

Fig. 8. Brush cytologic specimen obtained from the small intestine of a dog with moderate lymphocytic enteritis. Note the numerous small lymphocytes embedded within the raft of duodenal epithelia.

Fig. 9. Three color fluorescence in situ hybridization (FISH) image of a colonic biopsy specimen in a dog with inflammatory bowel disease. Cy-3 positive (orange) clostridia organisms are observed within a biofilm along with other FITC-labeled (green) bacteria adherent to the surface epithelia. DAPI-stained nuclei (blue) are also seen.

Fig. 10. Colonic biopsy specimen showing a diffuse infiltrate of periodic acid–Schiff (PAS) positive macrophages within the colonic mucosa of a boxer diagnosed with granulomatous colitis.

905

906 Fig. 11. Immunophenotyping performed on an ileal biopsy specimen of a cat diagnosed with GI
907 lymphoma. A dense homogenous (>90%) population of T lymphocytes (CD3+ T-cell stain) have
908 infiltrated within the ileal mucosa.

909

910 **Table 1.** Clinical indications and utility for endoscopic biopsy of the gastrointestinal tract

Endoscopic procedure	Clinical indications	Animal preparation	Diagnostic use	Specimen collection	Comments
Esophagoscopy	Signs of dysphasia/odynophagia, unexplained halitosis, nausea, regurgitation, coughing, anorexia, weight loss	Withhold food >12 h; radiograph for barium retention if contrast studies performed	Mucosal erosions, stricture, mass, foreign bodies	Endoscopic biopsy Cytologic specimens	Mucosal biopsy rarely performed except for masses or obvious infiltrates
Gastroscopy	Signs of vomiting, hematemesis, nausea, anorexia, weight loss	Withhold food >12 h; feed soft food as last meal before procedure; radiograph for barium retention if contrast studies performed	Mucosal friability, granularity, mass, ulcer/erosions, foreign bodies, pyloric mucosal hypertrophy, gastric nematodes	Endoscopic biopsy Cytologic specimens Parasite extraction	Good quality gastric biopsies are easy to obtain be sure to biopsy fundus, body, and antrum/pylorus
Duodenoscopy	Signs of small bowel diarrhea, melena, vomiting, anorexia, weight loss	Withhold food >12 h; feed soft food as last meal before procedure	Mucosal friability, granularity, mass, ulcer/erosions, foreign bodies	Endoscopic biopsy Cytologic specimens	Duodenal biopsies are quite friable
Ileoscopy	Signs consistent with either upper or lower GI disease	Withhold food >24 h; thorough colonic cleansing required	Mucosal friability, granularity, erosions	Endoscopic biopsy Cytologic specimens	Always obtain ileal biopsies; 'blind' forceps biopsies are OK
Colonoscopy	Signs of large bowel diarrhea, tenesmus, mucus, hematochezia	Withhold food >24 h; thorough colonic cleansing required	Mucosal friability, granularity, erosions, mass, vascular ectasia	Endoscopic biopsy Cytologic specimens	Always biopsy all three colonic regions

911

912 **Table 2**. Gastrointestinal diseases that may not have significant histopathologic abnormalities
913 (modified from Jergens et al., 2011)

GI diseases unaccompanied by significant histopathologic abnormalities
Motility disturbances
Brush border defects
Antimicrobial-responsive enteropathy
Secretory diarrheas
Adverse food reactions
Mucosal permeability defects

914 GI, gastrointestinal

915

916 **Table 3**. Definitions of endoscopic mucosal appearances

Mucosal appearance	Definition
Normal mucosa	No macroscopic lesions to mucosal surface
Friability	Bleeding on contact with endoscope or biopsy forceps
Granularity	Alteration in the texture of the mucosa
Erosion	Superficial linear mucosal defect(s) with hemorrhage
Hyperemia	Gradations of mucosal redness (pale → red)
Lymphatic dilatation	Multifocal to diffuse white foci within the mucosa
Mass	Abnormal growth of tissue projecting into lumen

917

918 **Table 4**. GIT endoscopic biopsy sample recommendations (Willard et al., 2008)

Species	Gastrointestinal organ	Number of endoscopic specimens [a]	Comments
Dog	Stomach	6 adequate	Biopsy gastric body unless focal lesions present
Dog	Duodenum	10-15 adequate	Up to 15 marginal samples may be required
Dog/cat	Ileum	3-5 adequate	Exact number unknown; blind forceps biopsies are OK
Dog/cat	Colon	9-12 adequate	Obtain 3-4 biopsies from each colonic region
Cat	Stomach	6 adequate	Six mucosal samples generally diagnostic
Cat	Duodenum	6 adequate	Six mucosal samples generally diagnostic

919
920 [a] Adequate refers to quality of endoscopic specimen i.e. diagnostically adequate
921

Table 5. Cucumber paper preparation for endoscopic sample submission (Swan and Davis, 1970)

Step	Instructions
1	Slice a firm cucumber as thinly as possible, avoiding seed areas
2	Place cucumber slices in 95% ethanol for 3 days; change ethanol daily
3	Then store the cucumber slices in 95% ethanol in a refrigerator
4	Remove endoscopic specimen from forceps and place on cumber slice in cassette. Do not allow the cucumber slices to dry out completely as specimens adhere less well to dry cucumber
5	Place cucumber-cassette unit into formalin container and submit to laboratory

Table 6. Qualitative assessment of mucosal appearance for endoscopic activity (Slovak et al., 2015)

Appearance	Score [a]	Description
Friability	0	Absent
	1	Present
Granularity	0	Absent
	1	Present
Erosions	0	Absent
	1	Present
Lymphatic Dilatation*	0	Absent
	1	Present

[a] Defined only during enteroscopy; Maximum gastroscopy score = 3; Maximum enteroscopy score = 4; Maximum colonoscopy score = 3