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Abstract
Cytologic examination of exfoliative specimens obtained during endoscopy is a useful and reliable adjunct to mucosal biopsy for the diagnosis of gastrointestinal (GI) tract diseases in dogs and cats. Clinical advantages of endoscopic cytology include simplicity, rapidity of diagnosis and minimal invasiveness. Cytologic smears are graded on the basis of objective criteria, including the presence and number of inflammatory, atypical, and epithelial cells as well as the presence of bacteria, hemorrhage, debris/ingesta, and mucus. There is high correlation between results obtained from endoscopic cytology and histologic examination, and discordant results are infrequent. Brush cytology is useful in detecting mucosal inflammation, whereas touch cytology is more likely to detect acute purulent and erosive mucosal lesions. Alimentary lymphoma may be readily diagnosed using either technique. This article provides an overview of how cytologic smears are prepared and evaluates their diagnostic accuracy.

Keywords
Veterinary Clinical Sciences, gastrointestinal tract, endoscopic cytology

Disciplines
Small or Companion Animal Medicine | Veterinary Medicine | Veterinary Pathology and Pathobiology

Comments
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Gastrointestinal Endoscopic Exfoliative Cytology: Techniques and Clinical Application

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ABSTRACT: Cytologic examination of exfoliative specimens obtained during endoscopy is a useful and reliable adjunct to mucosal biopsy for the diagnosis of gastrointestinal (GI) tract diseases in dogs and cats. Clinical advantages of endoscopic cytology include simplicity, rapidity of diagnosis, and minimal invasiveness. Cytologic smears are graded on the basis of objective criteria, including the presence and number of inflammatory, atypical, and epithelial cells as well as the presence of bacteria, hemorrhage, debris/ingesta, and mucus. There is high correlation between results obtained from endoscopic cytology and histologic examination, and discordant results are infrequent. Brush cytology is useful in detecting mucosal inflammation, whereas touch cytology is more likely to detect acute purulent and erosive mucosal lesions. Alimentary lymphoma may be readily diagnosed using either technique. This article provides an overview of how cytologic smears are prepared and evaluates their diagnostic accuracy.

Advances in endoscopy have revolutionized the detection of gastrointestinal (GI) tract diseases in companion animals. Histologic examination of forceps biopsies is used to establish a definitive diagnosis of mucosal disease. Endoscopic exfoliative cytology is a useful adjunct to biopsy for detection of GI tract diseases in humans and the results correlate highly with histologic observations in dogs and cats. However, little data exist that describe the findings made by using this diagnostic technique. This article reviews our experience using endoscopic exfoliative cytology in the diagnosis of canine and feline GI tract diseases.

COLLECTION METHODS AND SMEAR PREPARATION

Numerous mucosal disorders are amenable to diagnosis by endoscopic cytology (see Gastrointestinal Tract Disorders Amenable to Endoscopic Cytology). Cytologic specimens should be obtained after mucosal biopsy because this technique...
has shown the highest diagnostic accuracy for detecting GI tract diseases in humans.\textsuperscript{13,14} Cytologic smears may be prepared using the brush or touch technique.

With the brush technique, a single-use guarded cytology instrument is passed through the accessory channel of the endoscope and advanced to the mucosa. The brush is extended beyond its protective sheath and exfoliative specimens are obtained by rubbing the brush vigorously on the mucosa until slight hemorrhage occurs (Figure 1A). The brush is then retracted into its sheath and withdrawn from the endoscope. The brush is again extended from its sheath and rolled across a glass slide to make a cytologic smear (Figure 1B). The brush should be carefully rotated 360° while traversing the slide’s surface, thereby ensuring maximum transfer of cellular material (Figure 1C).

The touch technique involves the transfer of a mucosal specimen from the biopsy forceps to a glass slide using a hypodermic needle (Figures 2A and 2B). Multiple cytologic imprints can be made by placing a second slide on top of and at right angles to the first slide and applying light pressure (Figure 2C). Excessive downward pressure and smearing should be avoided because this may result in cell rupture. Ideally, four slides (two slides using each cytologic technique) should be prepared for each organ being evaluated en masse.\textsuperscript{16–18} A smear is prepared by drawing a second slide on top of and at right angles to the first slide and applying light pressure (Figure 2C). Multiple cytologic imprints can be made by placing a second slide on top of and at right angles to the first slide and applying light pressure. Excessive downward pressure and smearing should be avoided because this may result in cell rupture. Ideally, four slides (two slides using each cytologic technique) should be prepared for each organ being evaluated en masse.\textsuperscript{16–18}

**CRITERIA FOR CYTOLeGIC EXAMINATION**

Objective criteria for evaluation of GI tract exfoliative cytologic specimens have been described and validated.\textsuperscript{12} Briefly, a grading system based on several categories is used (Table I). Cytologic grades of 2 or lower for cellularity categories are generally not diagnostically significant. A minimum of 10 microscopic fields should be evaluated on each slide before a cytologic diagnosis is made. This grading system facilitates consistent and rapid evaluation of cytologic preparations (Figure 3).
Discordant results between cytologic and histologic findings were uncommonly observed. The percentages of false-positive results (11 of 314 paired cytologic specimens; 3.5%) and false-negative (24 of 314 paired cytologic specimens; 7.6%) results in our study were low and comparable to those of similar studies in humans.\textsuperscript{8,18,23} The reasons for discordance are diverse but include difficulty in differentiating mild inflammatory lesions from normal; technical complications caused by poor orientation of tissue during embedding for histologic examination or inadvertent sampling of a mucosal lymphoid aggregate; the presence of focal, fibrotic, or deeply infiltrative mucosal lesions; and the presence of functional GI tract disease.\textsuperscript{24}

**CLINICAL APPLICATIONS**

**Case 1: Chronic Postprandial Vomiting in a Dog**

**Clinical Synopsis**

A 13-year-old neutered poodle was referred because of 3 weeks of sporadic vomiting episodes that generally occurred 8 to 12 hours after eating. The vomitus frequently contained partially digested food and bile. Appetite and activity were normal, and no diarrhea or weight loss was reported. Physical examination revealed an alert, mildly obese dog.
normalities. An upper GI series using liquid barium demonstrated partial pyloric outflow obstruction. Upper GI tract endoscopy (i.e., gastroscopy, duodenoscopy) was performed.

**Endoscopic/Cytologic Observations**
Endoscopic examination revealed no abnormalities of the proximal stomach and gastric body. Within the antrum, a large polypoid mass was observed adjacent to the pylorus obstructing the pyloric opening. Brush cytologic specimens of the mass consisted of a uniform population of benign gastric epithelial cells with no evidence of malignancy (Figure 7). A cytologic diagnosis of epithelial hyperplasia was made. Owner consent was given for gastrotomy and the mass was successfully removed.

**Assessment**
Endoscopic cytology allowed a rapid intraoperative tentative diagnosis of epithelial hyperplasia. The client had requested humane euthanasia for her dog if gastric malignancy were detected; therefore, this facilitated a therapeutic decision of polypectomy. Histologic examination of the excised mass confirmed the cytologic findings (adenomatous polyp). Following surgery, vomiting episodes ceased and the dog was discharged.

**Case 2: Episodic Regurgitation in a Cat**

**Clinical Synopsis**
A 5-year-old neutered domestic shorthair cat was admitted for episodic regurgitation of 2 weeks’ duration. A moderately decreased appetite and weight loss (1 kg) were also reported by the owner. Physical examination revealed a thin, active cat with a fever (104˚F). Initial laboratory tests (i.e., complete blood count, serum biochemical profile, urinalysis, feline leukemia virus [FeLV] ELISA, cervical/thoracic radiography) revealed mild anemia (hematocrit, 28%), a positive FeLV test, and lateral displacement of the midcervical trachea. Ultrasound evaluation of the cervical region and an esophagram confirmed the presence of a periesophageal or esophageal wall mass. Esophagoscopy was performed the following day.
Endoscopic/Cytologic Observations
A large, smooth-surfaced, eroded mass occluding the proximal esophageal lumen was seen by esophagoscopy. The mass did not arise from the mucosa but projected into the lumen as a periesophageal structure. Cytologic specimens were obtained by both endoscopy and fine-needle aspiration of the mass under ultrasound guidance. Examination of cytologic preparations showed a homogenous population of large immature lymphocytes consistent with a diagnosis of lymphosarcoma (Figure 8). Histologic specimens of esophageal mucosa were nondiagnostic.

Assessment
Clinical observations and diagnostic testing were consistent with a diagnosis of primary esophageal disease. Exfoliative cytology allowed a diagnosis of periesophageal neoplasia to be made despite the absence of histologic confirmation of lymphosarcoma. This case emphasizes the adjunctive role of GI tract endoscopy in the diagnosis of alimentary neoplasia. The cat was treated with multiple-drug chemotherapy for lymphosarcoma, leading to rapid remission of clinical signs and radiographic lesions within 2 weeks. Clinical signs of regurgitation returned 9 months later, prompting euthanasia.

Case 3: Anorexia and Weight Loss in a Cat
Clinical Synopsis
A 12-year-old neutered domestic shorthair cat with a 1-year history of intermittent anorexia, vomiting, and weight loss was evaluated. Vomiting episodes were typically cyclic (occurring over 48 hours) and would then resolve. Weight loss exceeding 8 lb was confirmed. On physical examination, the cat was alert and active, but reduced lean muscle mass was noted. Over the previous 12 months, a variety of diagnostic tests had been performed in a step-wise fashion, including routine hematology, urinalysis, multiple serum total thyroxine tests, abdominal radiography, and serology for FeLV and feline immunodeficiency virus. Prophylactic dewormings and dietary trials resulted in little clinical improvement. Upper GI tract endoscopy was then performed.
Endoscopic/Cytologic Observations

Endoscopic examination of the esophagus and stomach was unremarkable. Visualization of the proximal duodenum revealed marked mucosal granularity and friability. Multifocal erosive lesions were apparent throughout most of the distal duodenum and jejunum. Small intestinal brush and touch cytologic specimens were similar with moderate-to-severe LP inflammation (Figure 9). Histologic examination of mucosal biopsy specimens confirmed the cytologic finding of LP enteritis (Figure 10). Gastric mucosal specimens were histologically normal.

Assessment

Severe LP enteritis was readily detectable using endoscopic exfoliative cytology. The magnitude of intestinal inflammation was marked as evidenced by a high (5 of 6) cytologic grading score, numerous sites of lymphocytic infiltration within the epithelium, and observation of large granular lymphocytes in brush specimens. Treatment of this cat included feeding a commercially prepared hypoallergenic diet and administration of prednisone and metronidazole at immunomodulating doses. Remission of signs occurred over 4 months, and drug administration was discontinued.

TABLE II
Distribution of Histologic Findings in Which Endoscopic Cytology Was Also Performed

<table>
<thead>
<tr>
<th>Histologic Finding</th>
<th>Stomach</th>
<th>Small Intestine</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>88</td>
<td>31</td>
<td>12</td>
</tr>
<tr>
<td>Spirochetes</td>
<td>3</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Inflammation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytic-plasmacytic</td>
<td>25</td>
<td>91+</td>
<td>18</td>
</tr>
<tr>
<td>Eosinophilic</td>
<td>4</td>
<td>3</td>
<td>NA</td>
</tr>
<tr>
<td>Mixed+</td>
<td>8</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Neoplasia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphosarcoma</td>
<td>3</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>1</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Atrophy/fibrosis</td>
<td>7</td>
<td>3</td>
<td>NA</td>
</tr>
<tr>
<td>Total cytologies+</td>
<td>139</td>
<td>135</td>
<td>40</td>
</tr>
</tbody>
</table>

NA = Not applicable.

*Includes inflammation seen with lymphangiectasia, bacterial overgrowth, and Physaloptera species infection.

**Denotes suppurative, eosinophilic, and/or granulomatous inflammation.

*Paired brush and touch cytologic specimens.

TABLE III
Diagnostic Accuracy of Cytologic Specimens Obtained During Endoscopy

<table>
<thead>
<tr>
<th>Value*</th>
<th>Stomach</th>
<th>Small Intestine</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>73</td>
<td>92</td>
<td>86</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>97</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>Positive predictive value (%)</td>
<td>91</td>
<td>92</td>
<td>100</td>
</tr>
<tr>
<td>Negative predictive value (%)</td>
<td>89</td>
<td>80</td>
<td>75</td>
</tr>
</tbody>
</table>

*Diagnostic indices calculated using standard formulas.
Case 4: Chronic Intermittent Vomiting in a Dog

Clinical Synopsis

A 1-year-old spayed Whippet was referred for endoscopic evaluation of chronic vomiting. Cyclic vomiting episodes over the preceding 4 months had been nonresponsive to dietary therapies and repeated dewormings. Diagnostic evaluation (i.e., hematology, serum biochemistries, urinalysis, fecal examinations, abdominal radiography) by the referring veterinarian showed no abnormal results. Upper GI tract endoscopy was performed.

Endoscopic/Cytologic Observations

Both esophagoscopy and gastroscopy failed to show mucosal abnormalities in this dog. In the proximal duodenum, several nematodes were observed along the mucosa, which also contained multifocal attachment sites. Extraction of two parasites confirmed them to be non-gravid female *Physaloptera* species. Mucosal specimens obtained for histologic/cytologic evaluation confirmed the presence of LP enteritis (Figure 11). Gastric biopsies were normal histologically.

Assessment

Brush cytology is extremely useful in detecting mucosal inflammation of various causes (Table II). Our experiences indicate that *Physaloptera* species infection is frequently accompanied by intense mucosal infiltrates of lymphoid cells that contribute to clinical signs.19 Treatment
of this dog with pyrantel (administered once) and metronidazole (to reduce mucosal cellular infiltrates) was curative.

Case 5: Tenesmus and Increased Frequency of Defecation in a Dog

Clinical Synopsis

A 1-year-old spayed boxer was referred for endoscopic evaluation of colonic disease. The animal had been straining to defecate and had shown an increased frequency of defecation for over 3 weeks. The feces were generally well formed but were encased by mucus. Physical examination, including thorough digital examination of the rectum, revealed no abnormalities. Initial diagnostic tests (i.e., hematology, serum biochemistries, urinalysis, survey abdominal radiography, and multiple fecal analyses) were unremarkable. The administration of multiple anthelmintic drugs also had failed to alleviate signs. Following preparation with an oral lavage solution, colonoscopy was performed.

Endoscopic/Cytologic Observations

Excellent cleansing with the lavage solution allowed full visualization of all mucosal surfaces. Most striking was the appearance of discrete nodules along the entire descending colonic mucosa, which was consistent with marked lymphoid hyperplasia. Similar, but fewer, lesions accompanied by multifocal erosions were observed in the transverse colon. Brush cytologic specimens showed moderate LP infiltrates in all colonic regions (Figure 12). Moderate to severe LP colitis was confirmed histologically from examination of mucosal biopsy specimens.
**Assessment**

Gastrointestinal tract endoscopic cytology provided a reliable tentative diagnosis while awaiting histologic review of biopsy specimens. Cytologic lesions were most pronounced in brush specimens, which is typical with mucosal inflammation. Specific therapy for this dog included dietary modification (low-residue diet) and drug therapy (immunomodulating doses of prednisone and metronidazole). The dog showed excellent response to therapy and clinical signs regressed over 6 weeks. Drugs were gradually tapered, and the dog has been maintained on diet alone.

**SUMMARY**

In conclusion, we believe that endoscopic cytology, which is a simple technique, is useful in the diagnosis of GI diseases in dogs and cats. The results of our prospective study indicate that the combined brush and touch cytology with mucosal biopsy under direct endoscopic visualization are useful in the diagnosis of GI inflammation and malignancy in a significant number of cases. We recommend that endoscopic cytology be routinely used as an adjunctive diagnostic technique whenever mucosal biopsy is performed.

**REFERENCES**

About the Authors
Drs. Jergens and Miles are affiliated with the Department of Veterinary Clinical Sciences and Dr. Andreasen with the Department of Veterinary Pathology, College of Veterinary Medicine, Iowa State University, Ames. Dr. Jergens is a Diplomate of the American College of Veterinary Internal Medicine, Dr. Andreasen is a Diplomate of the American College of Veterinary Pathologists, and Dr. Miles is a Diplomate of the American College of Veterinary Radiology.