**Diagnostic contribution of cytological specimens obtained from biopsies during gastrointestinal endoscopy in dogs and cats**

**Guillaume Ruiz[a, *], Lucas Verrot[a], Eve Laloy[b], Ghita Benchekroun[a]**

[a] Internal Medicine Department, Université Paris-Est, Ecole Nationale Vétérinaire d'Alfort, 7 avenue du général de Gaulle, 94704 Maisons-Alfort Cedex, France

[b] Pathology Department, Université Paris-Est, Ecole Nationale Vétérinaire d'Alfort, 7 avenue du général de Gaulle, 94704 Maisons-Alfort Cedex, France

[*] Corresponding author (tel. +44 7774720958). Current address for corresponding author is: Langford Veterinary Services-Small Animal Referral Hospital, University of Bristol, Langford House, Langford, BS40 5DU, United Kingdom

**Email addresses**

Guillaume Ruiz, DVM, MRCVS: g.ruiz@bristol.ac.uk

Lucas Verrot, DVM: lucas.verrot@hotmail.fr

Eve Laloy, DVM, dip-ECVP, PhD: elaloy@vet-alfort.fr

Ghita Benchekroun, DVM, dip-ECVIM-CA: gbenchekroun@vet-alfort.fr

**Acknowledgements**

The authors wish to thank Mr Loic Desquilbet for his assistance in doing the statistical analyses of the results.

Preliminary results were presented as an Abstract at the 25th European College of Veterinary Internal Medicine-Companion Animals (ECVIM-CA) Congress, Lisbon, 10-12 September 2015.

# Diagnostic contribution of cytological specimens obtained from biopsies during gastrointestinal endoscopy in dogs and cats


## Summary

**Objectives**: The aims of this study were to compare cytological samples obtained from endoscopic biopsies using ‘imprint’ and ‘squash’ techniques, and to evaluate the potential value of cytology compared to histology in reaching the diagnosis.

**Methods**: Eighteen dogs and five cats undergoing endoscopy for chronic gastrointestinal signs were prospectively included. Imprint and squash samples were obtained from one biopsy and then analysed. Comparison between cytology and histology was performed using Cohen’s kappa coefficient.

**Results**: Appropriate samples for cytological evaluation were most often obtained with the squash technique (96% of the cases vs. 68% with the imprint technique). The diagnoses obtained with cytological samples and by histology, considered as the gold standard, were compared. The same diagnosis was obtained with the squash technique in 65% of the cases. Furthermore, cytology was considered complementary to histology for gastric spiral organisms and mast cells identification.

**Clinical significance**: These results suggest that squash cytology obtained from endoscopic biopsies of the gastrointestinal tract could provide relevant and additional information to histology in dogs and cats. Further studies are needed to confirm these findings.



**Keywords:** cytology; endoscopy; gastrointestinal tract; imprint; squash

## Introduction

Endoscopy is commonly used in veterinary gastroenterology to assess macroscopic lesions of the mucosa and to obtain targeted samples (Washabau *et al*. 2010). Histology remains the current gold standard to achieve a definitive diagnosis for infiltrative and structural diseases. However, histological results are usually only available a few working days after endoscopy due to the time required for laboratory processing. Moreover, some abnormal findings such as organisms present within the surface mucus can be lost during the actual process and therefore misdiagnosed (Jergens *et al.* 1998).

Cytology is commonly used in veterinary medicine, and different techniques have been described (Cohen *et al*. 2003, Bonfanti *et al.* 2006, Ballegeer *et al*. 2007). However, lesions differ in their ability to exfoliate, thus creating a discrepancy between 'exfoliative lesions' (which often lead to a reliable cytological diagnosis) and 'poorly exfoliative lesions' (which are generally non-diagnosed with cytology) (Cohen *et al*. 2003).

Only a few studies have focused on the diagnostic contribution of cytological samples obtained from the digestive tract of dogs and cats (Tobey *et al.* 1988, Jergens *et al*. 1998, Bonfanti *et al*. 2006, Riondato *et al*. 2014, Mangelsdorf *et al*. 2015). The aims of this prospective study were to compare the abilities of 'imprint' and 'squash' techniques to provide valid cytological samples from biopsies obtained during gastrointestinal endoscopy in dogs and cats, and to evaluate the potential value of cytology compared to histology in reaching the definitive diagnosis.

## Materials and methods

This prospective study included dogs and cats presented for gastrointestinal symptoms and which underwent a gastrointestinal endoscopy between March 2012 and March 2013 at XXX Hospital. Cases were included only if biopsies, concurrent squash and imprint preparations for cytology, and a conclusive histopathology report, were available. Signalment and clinical signs were recorded.

The endoscopy was performed under general anaesthesia with a GIF-160 Olympus gastroscope, using FB-240K Olympus forceps for biopsies. At least five biopsies from each area of interest (i.e., stomach and proximal duodenum for upper alimentary tract, distal ileum and colon for lower alimentary tract) were collected in separate cassettes, fixed in 10% formalin and then routinely processed for histopathological analysis. An additional biopsy was used to obtain cytological specimens with two techniques. First, the 'imprint' specimen was obtained by imprinting the biopsy sample on a glass slide several times, using a 25 G needle to gently hold the sample. Then the 'squash' specimen was obtained by crushing the biopsy between two slides, and pulling them apart without smearing. The slides were then sent to the laboratory for cytological analysis, separately from the formalin jars.

The biopsies were stained with haematoxylin, eosin and saffron (HES), which are the routine stain used for all biopsies in our laboratory. They were reviewed by board certified pathologists from the Pathology Department of XXX, and interpreted according to the current guidelines of the WSAVA International Gastrointestinal Standardization Group (IGSG) described elsewhere (Day *et al*. 2008). The presence of organisms was also recorded. Biopsy samples of less than 3mm or without lamina propria were considered of insufficient quality and the cases were excluded.

Cytological samples were stained with May-Grünwald-Giemsa. All the slides were reviewed by a single ECVP-diplomate (XXX) in a blinded manner at the end of the recruitment process. The pathologist had access to the submission form (history and clinical signs) but was blinded to the histological results. The cytological samples were analyzed using a method adapted from Jergens *et al.* (1998) and Andreasen *et al.* (2009). The following categories were identified: inflammatory cells, atypical and neoplastic cells, epithelial clusters, gastric spiral organisms (GSO), bacterial flora, haemorrhage, debris/ingesta, and mucus. A cell count mean was calculated for each category from at least 10 fields in well spread areas of the slide, and was then classified in a grading system similar to that of Jergens *et al*. (1998) (Table 1). Samples with a grade 2 or less for epithelial clusters were considered non-representative of the organ and were thus classified as 'non

diagnostic'. Samples presenting poor preservation or numerous artefacts that precluded further interpretation - such as crushed cells - were also excluded from further analysis.

For each case, the diagnostic conclusions for both the cytological and histological analyses were classified into one of the categories described in Table 2, based on the predominant pathological findings, in order to allow comparison of the cytological and histological diagnoses. The cytological diagnosis obtained by either the imprint or squash technique, as compared with histopathological analysis (considered as the gold standard) was assessed by statistical analysis. The agreement between cytology and histology was determined by Cohen's Kappa coefficient. The techniques were considered in agreement if both concluded to the exact same diagnosis, as per Table 2. The different kappa ($\kappa$) values were scored as follows: very poor for $\kappa<0$, poor for $0<\kappa<0.2$, fair for $0.21<\kappa<0.4$, moderate for $0.41<\kappa<0.6$, good for $0.61<\kappa<0.8$ and excellent for $0.81<\kappa<1$ (Landis & Koch 1977).

## Results

Twenty-three cases, including 18 dogs and five cats, met the inclusion criteria. The ages ranged from one to 16 years (from one to 13 years in dogs and from four to 16 years in cats). There were eight females (seven dogs and one cat) and 15 males (11 dogs and four cats). All the cats were domestic shorthairs, whereas the dogs were of various breeds: three French Bulldogs, three Jack Russell Terriers, three crossbreeds, two German Shepherds, one Labrador Retriever, one Rhodesian Ridgeback, one Boxer, one Weimaraner, one Parson Terrier, one Lhasa Apso and one West Highland White Terrier.

Clinical presentation was variable but included at least one of the following signs: vomiting (17 cases), diarrhoea (seven cases), inappetence (five cases), weight loss (five cases), lethargy (four cases), haematochezia (two cases), retching (two cases), melaena (one case), tenesmus (one case), and constipation (one case).

Eighteen cases had biopsies taken from the stomach (fundus in 11 cases, antrum in one case, and both fundus and antrum in six cases). Seventeen cases had biopsies taken from the small intestine (duodenum only in 13 cases, ileum only in three cases and both duodenum and ileum in one case). Six cases had biopsies taken from the large intestine (colon in five cases and rectum in one case). In total, 48 sites were collected during the course of the study. Histopathological analysis resulted in 24 diagnoses because one case was diagnosed with two distinct conditions; they are presented in Table 3 (see online).

For the cytological evaluation, 95 specimens were examined (48 slides obtained with the squash technique, and 47 slides with the imprint technique - one slide of small intestine was lost during the laboratory processing). Only 4% of the cytological samples obtained by squash technique (2/48) were considered as 'non diagnostic', based on an insufficient number of epithelial cell clusters, as described above. For the imprint technique, 32% of the samples (15/47) were considered as 'non diagnostic'. All diagnostic specimens obtained with the imprint technique (32/47) were also diagnostic with the squash technique. All 'non diagnostic' samples were excluded from further statistical evaluation, in order to focus on the agreement between cytology and histopathology when sample quality was good enough to reach a diagnosis.

Squash cytology and histology gave the same results in 65% of the cases (30/46). Agreement between the two techniques was considered 'moderate' ($\kappa$=0.48 [0.32; 0.65]). This agreement was 'fair' for the imprint technique ($\kappa$=0.39 [0.2; 0.58]). Squash and imprint techniques led to the same cytological result in 84% of the 32 specimens for which imprint cytology was diagnostic. The conclusion reached for the remaining specimens (5/32) with the imprint technique was 'within normal limits', whereas it was 'abnormal' with the squash technique. The overall agreement between the two cytological techniques was considered 'good' ($\kappa$=0.75 [0.52; 0.98]). The agreements calculated for each part of the digestive tract are summarized in Table 4.

Organisms were observed in nine cases. Gastric spiral organisms were found in two cases on both the histological and squash samples, in three cases on histological samples only, and in three cases on squash samples only (Fig. 1A and 1B). They were also observed in three cases on imprint samples, and the squash technique was also diagnostic for each of them. In one dog, the histological analysis revealed amastigotes forms of *Leishmania* spp. in the stomach, ileum and colon, whereas the two cytology techniques only revealed them in colonic samples.

The cases with gastric samples were retrospectively reviewed for presence of mast cells. These cells were never observed on the HES-stained histological samples, as gastrointestinal mast cells can be more difficult to identify with conventional HES-stain (Ramsay *et al.* 2010). Conversely, mast cells were found on cytological samples obtained from 4/5 cases where GSO had been diagnosed by cytology (at a level of one cell per field at x400 magnification) (Fig. 1A). Mast cells were also found in another case for which GSO had only been detected by histology. Mast cells were never found in cases other than those diagnosed with GSO.

## Discussion

In 1998, Jergens *et al*. described perendoscopic techniques (cytobrush and biopsy imprint) to obtain cytological smears. They reported that the accuracy of cytology was satisfactory and they recommended taking samples for cytology in adjunction to biopsies during endoscopy. Eight years later, Bonfanti *et al.* (2006) reported that direct impression smears of biopsies obtained from gastrointestinal lesions appeared to be more accurate than ultrasound-guided FNA in diagnosing digestive tumours by cytological examination. More recently, Riondato *et al*. (2014) demonstrated the reliability of cytological examination of squash preparations from endoscopic gastric biopsies to diagnose canine gastric adenocarcinomas.

Our study demonstrated that the cytological smears obtained by ‘squash’ technique were of better quality than those obtained by ‘imprint’ technique (4% vs. 32% of non diagnostic samples due to insufficient quality, respectively). Furthermore, in all cases for which the imprint samples agreed with the histological diagnosis, the same diagnosis was obtained with the squash technique. The good quality of squash samples for cytology has also been reported in another recent study (Mangelsdorf *et al*. 2015). The discrepancy between the two might be explained by the actual technique. When an imprint smear is prepared, the biopsy sample is applied several times on a slide, but leads only to the deposition of superficial cells from the sample. With the squash technique, the sample is crushed on the slide, allowing cells from deeper layers of the biopsy to be deposited on the slide. However, the clinician must pay attention to the thickness of the smear and avoid cell mounds that would be uninterpretable for the pathologist.

The overall agreement between cytology (with the squash technique) and histopathology was 65%, which was considered moderate ($\kappa$=0.48 [0.32; 0.65]) and was consistent with a previous comparative study of cytology and histology techniques (Cohen *et al.* 2003). The terminology associated with kappa’s values is derived from a study by Landis and Koch (1977), but this terminology could be misleading to the clinician. Due to the small number of cases included in our study, it is hard to say whether some diseases are more likely to be identified than others with cytology. Nevertheless, only one case led to a significant disagreement, where the conclusion with squash cytology was neutrophilic gastritis, whereas it was diagnosed as lymphoma with histopathology, and the imprint technique was inconclusive. Although lymphoma can usually be identified by cytology due to the ability of neoplastic cells to exfoliate, in our case, the squash sample might only have caught the inflammatory reaction associated with the tumour (Bonfanti *et al.* 2006). Regarding other cases of disagreement, cytology appeared normal or the conclusion was an inflammatory process, as with histopathology, but a different cell population was identified (eosinophilic instead of lymphoplasmacytic, lymphoplasmacytic instead of neutrophilic, and lymphoplasmacytic instead of eosinophilic, respectively with cytology and histology, in one case

each). In conclusion, squash cytology appears interesting as a first diagnostic approach, but histopathology remains necessary. Considering that cytology is generally faster than histology to obtain results, squash samples may help the clinician to initiate an appropriate treatment more rapidly, especially in the case of neoplastic disease such as lymphoma.

For the small intestine, histology and squash cytology led to the same diagnosis in 82% of the 17 cases. Cytology also gave the best diagnostic agreement at this site in another study (Jergens *et al.* 1998). Paradoxically, the agreement between the two techniques in this particular location, as calculated by the kappa coefficient, was poor ($\kappa$=0.15 [-0.07; 0.38]). In our study lymphocytic-plasmacytic enteritis, as demonstrated in Fig. 2A and 2B, was the condition most commonly diagnosed by histology (n=13/17) and was often correctly identified by cytology (n= 9/12 for the imprint technique and n=13/13 for the squash technique) (Table 3 – see online). However other less commonly encountered conditions (such as eosinophilic enteritis or non inflammatory fibrosis) were not correctly identified by cytology (n= 0/4 for both imprint and squash techniques). The disagreement between cytology and histology in diagnosing such conditions explains the low value of the kappa coefficient. A future study focusing on inflammatory bowel disease could be useful to confirm the diagnostic contribution of a cytological examination using squash preparations obtained from gastro-intestinal biopsies in this particular situation.

In three cases, gastric spiral organisms were only observed in squash cytology, and were not observed in the corresponding histological slides. It is unlikely that these organisms were initially absent from the histological samples because both biopsies for cytological and histological examinations were taken from the same area of the stomach. The potential of cytology in detecting GSO has already been reported in other studies, and different explanations can be proposed (Happonen *et al.* 1996, Jergens *et al.* 1998). First, spiral bacteria are found in the surface mucus of the stomach. This mucus is likely to be eliminated during manipulation of the histological sample, leading to organism loss. Secondly, special stains (such as Warthin-Starry stain) are generally

198 required to recognize GSO because spiral organisms are poorly stained with HES. Conversely, they
199 are easily seen with the standard cytological stain (May, Grunwald and Giemsa). To conclude and
200 providing this finding is confirmed in a larger cohort of cases, it appears that cytology can help in
201 detecting *Helicobacter* infection in the cases where these organisms are missed on histology.

202 In our study, mast cells were observed in 5 cases of gastritis. They were only detected by cytology
203 and never by histology. This was expected, as mast cells in the gastrointestinal tract are usually well
204 stained with conventional cytological stains whereas their identification by histopathology can be
205 more challenging with conventional HE stains and requires specific stains (e.g., Giemsa, toluidine
206 blue) (Ramsay *et al.* 2010). More interestingly, we noticed a close association between the presence
207 of mast cells and GSO. In human medicine, mast cells are often seen in the gastric mucosa in
208 association with *Helicobacter pylori*, and recent studies have documented their involvement in the
209 initiation and promotion of mucosal oedema, attraction of neutrophils within the mucosa and
210 epithelial cells apoptosis (Nakajima *et al.* 2004, Hofman *et al*. 2007, Caruso *et al*. 2011). Further
211 studies focusing on this association are needed, as they could contribute to a better understanding of
212 the pathologic role - if any - of GSO in chronic gastritis in dogs and cats.

213 Neoplastic processes were diagnosed in five cases by histopathology (high-grade lymphoma in four
214 cases, as demonstrated in Fig 3B, and colonic adenoma in one case) (Table 3 – see online).
215 However, only two lymphomas were identified by cytology (both imprint and squash techniques, as
216 demonstrated in Fig 3A). For the other cases, cytological analysis was either not conclusive or led
217 to a false diagnosis (neutrophilic gastritis in one case, and carcinoma in another case). This was
218 unexpected, as round cell tumours such as lymphomas are usually considered as 'well-exfoliating'
219 lesions. Similarly, the sensitivity of cytology (squash smear technique) to diagnose low-grade
220 alimentary lymphoma was low in a recent study (Mangelsdorf *et al.* 2015). However, a better
221 sensitivity is expected to diagnose high-grade alimentary lymphoma. Indeed, in the study by
222 Bonfanti *et al*. (2006), both sensitivity and specificity were scored as 100% when diagnosis of
223 gastrointestinal lymphoma was based on cytological examination of impression smears (obtained

from surgical or post-mortem biopsies). In our study, cytological samples were obtained from a single endoscopic biopsy. This probably explains the poor diagnostic contribution of cytology in cases of lymphoma in our study. A further study, focused on lymphoma cases and using more biopsies to obtain squash preparations, will be required to confirm this hypothesis.

Our study has several limitations. The small number of cases and the diversity of the diagnoses make generalization difficult. In particular, further studies involving a larger number of patients could be useful to see if some diseases are more likely to be diagnosed by cytology than others. Only one single biopsy was collected for both cytological techniques, and the squash specimen was always obtained after performing the imprint specimen. It is possible that this had a negative influence on the quality of the squash specimen. Different pathologists were involved for interpretation of histopathological slides, and although they all followed the current published guidelines (Day *et al.* 2008), this limited the standardization of the results.

The primary aim of our study was to obtain preliminary results regarding the interest of cytology compared to histology. For this reason, we performed only one slide for each cytology technique to limit the number of additional biopsies taken from the patients, whereas histological diagnosis was based on at least five biopsy samples from each area. Obviously, increasing the number of cytological samples is likely to improve the agreement rate between cytology and histology and this could be investigated further based on the results of the current preliminary study.

Our study included no ‘control population’ of healthy dogs and cats, for ethical reasons. This is certainly a limiting factor in comparing the sensitivity and specificity of cytology and histology of the gastrointestinal tract. However, the purpose of this study was mainly to identify the value of cytology as an adjunct to histology, considered as the gold standard to achieve a definitive diagnosis for structural diseases.

## Conclusions

This pilot prospective study shows that cytological examination of squash preparation obtained from endoscopic gastrointestinal biopsies can be of interest in gastrointestinal disease investigation. The technique is easy, quick and cost-effective to perform, and provides preliminary results which can help the clinician to initiate a treatment pending the histopathological analysis. Furthermore, cytology may be a useful adjunct for finding organisms (i.e., GSO). Although these are only preliminary results, further studies focusing on the squash technique and a larger number of cases would be of interest to determine more accurately the place of cytology in gastrointestinal disease diagnosis in dogs and cats.

## Conflict of interest statement

The authors disclose no conflict of interest. None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

## References


Andreasen, C.B., Jergens, A.E., Meyer, D.J. (2009) Oral cavity, gastrointestinal tract, and associated structures. In: Canine and feline cytology, A color atlas and interpretation guide. 2nd edn. Eds R.E. Raskin and D.J. Meyer. Saunders, Philadelphia. pp 207-229

Ballegeer, E.A., Forrest, L.J., Dickinson, R.M., *et al.* (2007) Correlation of ultrasonographic appearance of lesions and cytologic and histologic diagnoses in splenic aspirates from dogs and cats: 32 cases (2002-2005). *Journal of American Veternary Medical Association* 230, 690-696

Bonfanti, U., Bertazzolo, W., Bottero, E., *et al.* (2006) Diagnostic value of cytologic examination of gastrointestinal tract tumors in dogs and cats: 83 cases (2001-2004). *Journal of American Veterinary Medical Association* 229, 1130-1333

Caruso, R.A., Parisi, A., Crisafulli, C., *et al*. (2011) Intraepithelial infiltration by mast cells in human *Helicobacter pylori* active gastritis. *Ultrastructural Pathology* 35, 251-255

Cohen, M., Bohling, M.W., Wright, J.C., *et al.* (2003) Evaluation of sensitivity and specificity of cytologic examination: 269 cases (1999-2000). *Journal of American Veterinary Medical Association* 222, 964-967

Day, M.J., Bilzer, T., Mansell, J., *et al.* (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 Comparative Pathology* 138, S1-S43

Happonen, I., Saari, S., Castren, L., *et al.* (1996) Comparison of diagnostic methods for detecting gastric *Helicobacter*-like organisms in dogs and cats. *Journal of Comparative Pathology* 115, 117-127

283 Hofman, V., Lassalle, S., Selva, E., *et al.* (2007) Involvement of mast cells in gastritis caused by
284 *Helicobacter pylori*: a potential role in epithelial cell apopotosis. *Journal of Clinical Pathology* 60,
285 600-607
286 Jergens, A.E., Andreasen, C.B., Hagemoser, W.A., *et al.* (1998) Cytologic examination of
287 exfoliative specimens obtained during endoscopy for diagnosis of gastrointestinal tract disease in
288 dogs and cats. *Journal of American Veterinary Medical Association* 213, 1755-1759

289 Landis, J.R., Koch, G.G. (1977) The measurement of observer agreement for categorical data.
290 *Biometrics* 33, 159-174

291 Mangelsdorf, S., Teske, E., v. Bomhard, W., *et al*. (2015) Cytology of endoscopically obtained
292 biopsies for the diagnosis of chronic intestinal diseases in cats. *Tierärztliche Praxis Kleintiere* 43,
293 15-22

294 Nakajima, S., Bamba, N., Hattori, T. (2004) Histological aspects and role of mast cells in
295 *Helicobacter pylori*-infected gastritis. *Alimentary Pharmacology and Therapeutics* 20, 165-170

296 Ramsay, D., Stephen, S., Borum, M., et al. (2010) Mast cells in gastrointestinal disease.
297 *Gastroenterology & hepatology* 6, 772-777

298 Riondato, F., Miniscalco, B., Berio, E., *et al.* (2014) Diagnosis of canine gastric adenocarcinoma
299 using squash preparation cytology. *The Veterinary Journal* 201, 390-394

300 Tobey, J.C., Willard, M.D., Krehbiel, J.D. (1988) Comparison of cytologic and histopathologic
301 evaluations of duodenal biopsies (abstr). *Veterinary Clinical Pathology* 18, 13
302 Washabau, R. J., Day, M. J., Willard, M. D., *et al.* (2010) Endoscopic, biopsy, and histopathologic
303 guidelines for the evaluation of gastrointestinal inflammation in companion animals. *Journal of*
304 *Veterinary Internal Medecine* 24, 10-26

**Figure legends**

Fig. 1A. Stomach, dog. Gastric spiral organisms (arrowheads) and a mast cell (arrow). The strands of eosinophilic material (chromatin) in the background are artefacts. Cytology, squash preparation, May-Grünwald-Giemsa stain. Bar = 20 µm. Insert: detail of spiral organisms at higher magnification.

Fig. 1B. Mucosa, stomach, same dog as Fig. 1A. Gastric spiral organisms in the lumen of a crypt. Histology, Warthin-Starry stain. Bar = 20 µm.

Fig. 2A. Stomach (pylorus), dog. Several small lymphocytes (arrowheads) close to epithelial cell clusters (arrows): chronic lymphocytic gastritis. Cytology, squash preparation, May-Grünwald-Giemsa stain. Bar = 20 µm.

Fig. 2B. Mucosa, stomach (fundus), same dog as in Fig. 2A. Increased number of lymphocytes and plasma cells in the lamina propria: chronic lymphoplasmacytic gastritis. Histology, haematoxylin-eosin-saffron stain. Bar = 20 µm.

Fig. 3A. Stomach (pylorus), dog. Large lymphoblasts predominate: gastric lymphoma. Cytology, squash preparation, May-Grünwald-Giemsa stain. Bar = 20 µm.

Fig. 3B. Mucosa, stomach (pylorus), same dog as in Fig. 3A. Large lymphoblasts infiltrate the lamina propria and the lamina muscularis: gastric lymphoma. Histology, haematoxylin-eosin-saffron stain. Bar = 20 µm.

**Table 1**

Grading system used for cytological analysis. Adapted from Jergens et al. (1998) and Andreasen et al. (2009).

| Categories | Description | Definition of grades used | |
|---|---|---|---|
| Inflammatory cells | Neutrophils, eosinophils, lymphocytes, plasma cells, macrophages, mast cells | Grade 0: no cell<br>Grade 1: 1 cell<br>Grade 2: 2 cells<br>...<br>Grade 7: ≥7 cells | Per field at 40x-Objective |
| Atypical/neoplastic cells | | | |
| Gastric Spiral Organisms (GSO) | | Grade 0: absence<br>Grade 1 to 2: presence of mild amount<br>Grade 3 to 4: presence of moderate amount<br>Grade 5 to 7: presence of marked amount | |
| Bacterial flora | Rods and cocci | | |
| Haemorrhage | Presence of peripheral blood | | |
| Debris/ingesta | Plant material, darkly pigmented particulate matter | | |
| Mucus | Diffusely basophilic mucinous material or rounded mucinous globules | | |
| Epithelial clusters | | Grade 0: none<br>Grade 1: 1 to 2 clusters<br>Grade 2: 3 to 4 clusters<br>Grade 3: 4 to 5 clusters<br>Grade 4: 6 to 7 clusters<br>Grade 5: 7 to 8 clusters<br>Grade 6: 9 to 10 clusters<br>Grade 7: > 10 clusters | Per field at 10x-Objective |

**Table 2**

Diagnostic categories defined for both cytological and histological analyses to allow comparison of the techniques.

| | |
|---|---|
| Normal | |
| Inflammation | Lymphoplasmacytic |
| (defined by the predominant cell population) | Eosinophilic |
| | Neutrophilic |
| | Histiocytic |
| Non inflammatory fibrosis | |
| Neoplasia | Lymphoid |
| | Epithelial |
| | Mesenchymal |
| | Neuroendocrine |

**Table 3**

Histological and cytological results obtained for the 23 cases included in the study.

| Species | Breed | Sex | Age | Histological diagnosis | Cytological diagnosis | |
|---|---|---|---|---|---|---|
| | | | | | Imprint | Squash |
| Cat | DSH[1] | Male | 11 | Gastric lymphoma | Gastric lymphoma | Gastric lymphoma |
| | DSH | Male | 11 | Rectal lymphoma | Non conclusive | Non conclusive |
| | DSH | Male | 16 | Gastric lymphoma | Non conclusive | Neutrophilic gastritis |
| | DSH | Female | 12 | Lymphoplasmacytic gastroenteritis | Eosinophilic gastritis and lymphoplasmacytic enteritis | Eosinophilic gastritis and lymphoplasmacytic enteritis |
| | DSH | Male | 4 | Lymphoplasmacytic gastritis + GSO[2] | Non conclusive | Normal stomach + GSO |
| Dog | West highland white terrier | Male | 13 | Lymphoplasmacytic enteritis | Slide not available | Lymphoplasmacytic enteritis |
| | French bulldog | Female | 3 | Neutrophilic gastritis + GSO, normal duodenum | Normal stomach, lymphoplasmacytic enteritis | Normal stomach, lymphoplasmacytic enteritis |
| | Lhasa apso | Female | 5 | Normal stomach, lymphoplasmacytic enteritis, normal colon | Normal | Normal stomach, lymphoplasmacytic enteritis, normal colon |
| | Jack Russell terrier | Male | 10 | Gastric lymphoma, lymphoplasmacytic enteritis | Gastric lymphoma, lymphoplasmacytic enteritis | Gastric lymphoma, lymphoplasmacytic enteritis |
| | Rhodesian ridgeback | Female | 10 | Colonic adenoma | Non conclusive | Colonic carcinoma |

[1] DSH: domestic shorthair
[b] GSO: gastric spiral organisms

| | | | | | |
|---|---|---|---|---|---|
| Parson terrier | Male | 12 | Lymphoplasmacytic gastroenteritis | Stomach non conclusive, lymphoplasmacytic enteritis | Normal stomach + GSO, lymphoplasmacytic enteritis |
| Labrador | Female | 1 | Lymphoplasmacytic gastroenteritis + *Leishmania spp.* amastigotes | Stomach non conclusive, lymphoplasmacytic enteritis + *Leishmania spp.* amastigotes | Normal stomach, lymphoplasmacytic enteritis + *Leishmania spp.* amastigotes |
| Labrador | Male | 4 | Lymphoplasmacytic gastroenteritis + GSO | Normal stomach, lymphoplasmacytic enteritis | Lymphoplasmacytic gastroenteritis |
| German shepherd | Male | 9 | Lymphoplasmacytic enteritis | Normal | Lymphoplasmacytic enteritis |
| Boxer | Male | 10 | Normal stomach, lymphoplasmacytic enteritis (small and large intestine) | Stomach non conclusive, lymphoplasmacytic enteritis, normal colon | Stomach non conclusive, lymphoplasmacytic enteritis (small and large intestine) |
| Crossbred | Male | 3 | Lymphoplasmacytic gastritis + duodenal fibrosis | Normal stomach + GSO, lymphoplasmacytic enteritis | Lymphoplasmacytic gastroenteritis + GSO |
| German shepherd | Male | 7 | Normal stomach, eosinophilic enteritis | Normal stomach and duodenum | Normal stomach and duodenum |
| Weimaraner | Female | 12 | Normal stomach, lymphoplasmacytic enteritis | Stomach non conclusive, lymphoplasmacytic enteritis | Normal stomach, lymphoplasmacytic enteritis |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Crossbred | Female | 11 | Eosinophilic enteritis + colonic fibrosis | Ileum non conclusive, normal colon | Lymphoplasmacytic enteritis, normal colon |
| | French bulldog | Male | 3 | Normal stomach +GSO, lymphoplasmacytic enteritis | Normal stomach + GSO, lymphoplasmacytic enteritis | Normal stomach + GSO, lymphoplasmacytic enteritis |
| | French bulldog | Female | 7 | Neutrophilic gastritis + GSO and lymphoplasmacytic enteritis | Stomach non conclusive, normal duodenum | Normal stomach, lymphoplasmacytic enteritis |
| | Jack Russell terrier | Male | 11 | Lymphoplasmacytic gastroenteritis | Normal stomach, lymphoplasmacytic enteritis | Normal stomach, lymphoplasmacytic enteritis |
| | Jack Russell terrier | Male | 12 | Lymphoplasmacytic gastritis | Lymphoplasmacytic gastritis + GSO | Lymphoplasmacytic gastritis + GSO |

**Table 4**

Agreement between cytological techniques and histology for the different parts of the digestive tract.

| Organ | Technique | Number of specimens | Number of specimens considered 'diagnostic'[1] | Number of agreements between cytology and histology[2] | Kappa value |
|---|---|---|---|---|---|
| Stomach | Imprint | 24 | 13 | 7 | 0.42 [0.18; 0.65] |
| | Squash | 24 | 23 | 12 | 0.37 [0.18; 0.56] |
| Small intestine | Imprint | 17[3] | 15 | 10 | 0.04 [-0.3; 0.37] |
| | Squash | 18 | 18 | 14 | 0.15 [-0.07; 0.38] |
| Large intestine | Imprint | 6 | 4 | 2 | 0.27 [-0.24; 0.78] |
| | Squash | 6 | 5 | 4 | 0.72 [0.23; 1.22] |
| Total | Imprint | 47 | 32 | 19 | 0.39 [0.2; 0.58] |
| | Squash | 48 | 46 | 30 | 0.48 [0.32; 0.65] |

[1] Number of specimens of sufficient quality to be interpretable
[2] Amongst 'diagnostic specimens'
[3] One of the 18 imprint specimens was lost during laboratory process