Evaluation of the quality of endoscopically obtained esophageal biopsies in the dog

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Key words
Gastroscopy, canine, specimen, gastro-esophageal junction, histology, morphometry

Summary
Objective: An important premise for obtaining diagnostically relevant histology specimens is an appropriate biopsy technique. Goal of this study was to determine if biopsies of adequate quality can be obtained from the canine esophagus at the gastro-esophageal junction (GEJ) during routine upper gastro-intestinal endoscopy. Material and methods: Over the course of one year, 58 dogs undergoing upper gastrointestinal endoscopy because of the presence of esophageal (n = 22) or gastrointestinal (n = 36) clinical signs were prospectively included. Five biopsies were repeatedly collected from the same dorsal and ventral locations of the GEJ, fixed individually in 4% neutral buffered formaldehyde, and evaluated histopathologically after standard preparation and haematoxylin and eosin staining. The presence of esophageal squamous epithelium with a basal cell layer and lamina propria mucosae in conjunction with foveolar columnar epithelium and cardiac glands, and the absence of fundic glands in one specimen, respectively, was judged as an adequately sampled biopsy. Results: Adequately sampled biopsies were reported in 45 out of 58 dogs, with 31 samples originating from the dorsal GEJ, 36 samples originating from the ventral GEJ, and with 22 samples originating from both sites, respectively. The incidence of adequately sampled biopsies increased significantly over time (r = 0.22; p < 0.05), with these biopsies being reported significantly more often during the last 6 months compared to the first 6 months of the study (p = 0.03). Histopathological evaluation of the esophageal squamous epithelium showed fibrosis, inflammation, elongation of the stromal papillae, and increased thickness of the basal cell layers in 14 out of 58 dogs. Stromal papillae of the ventral esophageal epithelium were significantly elongated in dogs with esophageal clinical signs compared to dogs with gastrointestinal clinical signs (p = 0.03). Conclusion and clinical relevance: After an initial learning phase adequate esophageal biopsies from the GEJ can be obtained in canine patients undergoing upper gastrointestinal endoscopy, and histological lesions can be found in these biopsies.

Bewertung der Qualität endoskopisch gewonnener Ösophagusbiopsate beim Hund

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Introduction

Over the last decades, endoscopy of the canine esophagus, stomach and duodenum has become a useful routine diagnostic technique in specialized veterinary centres, and taking biopsies from the stomach and duodenum has become a standard in canine upper gastrointestinal endoscopy (4, 27, 34, 40, 43, 47). In contrast esophageal biopsies are not routinely taken during upper gastrointestinal endoscopy. This might be due to the esophageal squamous epithelium's tight connection to the lamina propria. Helpful biopsy systems such as suction capsules or rigid forceps are less practicable for routine use (13, 15, 18). Using standard biopsy forceps, further preparation und histopathological judging may be challenging because of quality limitations of specimens obtained in such a manner (28). With the exception of canine esophageal cancer only few indications seem to warrant esophageal biopsies in dogs. This is why potential associations between endoscopic esophageal findings and histopathologic abnormalities are unknown in veterinary medicine (13, 15, 17, 18).

One of the most common gastro-intestinal disorders in humans is gastro-esophageal reflux disease (41), which is characterized by the presence of esophageal symptoms like heartburn and regurgitation, and esophageal lesions such as esophagitis and intestinal metaplasia (2, 12, 16). In dogs the true incidence of this disorder is unknown. Pathological gastro-esophageal reflux has been implicated as the causative event for clinical signs, as well as esophageal or laryngeal lesions in dogs (1, 11, 18, 20, 25, 26, 29, 32). Esophageal lesions develop when gastric acid or digestive enzymes reach the esophagus through the lower esophageal sphincter challenging and compromising the esophageal squamous epithelium's physiologic protecting mechanisms such as the sphincter function, esophageal clearance mechanisms, and the natural resistance of the mucosal barrier repeatedly or over prolonged periods of time (8, 9, 36, 38). However, it should be noted that intraesophageal pH has never been measured in any of the reported cases, and the proof of concept is still lacking in dogs. A recent study evaluating esophageal pH in dogs presenting with clinical signs commonly interpreted as gastroesophageal reflux using wireless ambulatory intraesophageal pH monitoring failed to document relevant reflux episodes (23). In the dog structural lesions caused by pathological gastro-esophageal reflux such as inflammation, erosion, elongation of the stromal papillae, and increased thickness of the basal cell layers can be induced experimentally (9, 30). Such lesions have also been detected in naturally diseased dogs, but have been rarely identified in endoscopic biopsies sampled from the esophagus (12, 16–18). The distribution of these lesions within the esophagus is unknown in the dog. Because gastric refluxates extend from the stomach through the cardia into the esophagus, structural lesions are rather to be expected in biopsy specimens obtained circumferentially from the gastro-esophageal junction (7, 19, 46). Biopsies obtained from this site are characterized by the concurrent presence of esophageal and cardiac mucosa. In the dog sampling of specimens adequate for histological judging is possible but may be associated with methodical difficulties (28).

Goal of this study was to test the adequacy of an esophageal biopsy technique during routine upper gastrointestinal endoscopy. It was hypothesized that adequate biopsies can be consistently collected from the esophagus at the canine gastro-esophageal junction, and that histological lesions are identifiable in adequately obtained esophageal biopsy specimens.

Materials and methods

A total of 58 dogs presented to a referral practice between July 2011 and June 2012 with an indication for upper gastrointestinal endoscopy due to the presence of esophageal or gastrointestinal clinical signs were prospectively included into the study. All data from the upper gastrointestinal endoscopies were collected by using a standard form encompassing signalement, patient history, clinical signs, and endoscopic findings.

Esophagoscopy and biopsy

All endoscopies were performed by the same operator using a paediatric fiberscope (Olympus GIF-PQ20) with patients in a left lateral recumbency and under general anaesthesia. After passing through the upper and lower esophagus the hiatus of the dia-
phragn was identified, the cardia was passed, and subsequently the endoscope was pulled back slowly to the gastro-esophageal junction. The tip of the endoscope was placed in such a manner, that the dorsal esophagus and the cardia of the greater curvature were visible to the right side, and the ventral esophagus and the cardia of the lesser curvature were visible to the left side of the picture endoscopically projected, respectively. The targeted biopsy site was the gastro-esophageal junction, characterized by a change from the bright colour of the esophageal squamous epithelium to the red colour of the cardiac foveolar epithelium (Fig. 1).

Five biopsies were collected from the gastro-esophageal junction during each gastroscopy: first from the junction composed of the dorsal esophagus and the cardia of the greater curvature, and then from the junction composed of the ventral esophagus and the cardia of the lesser curvature. Biopsying was performed by application of standard forceps for single use (diameter 2.3 mm, open elliptic branches, and a lancet: MTW-Endoskopie®, Wesel, Germany; Artikel-Nr. 99063502815) as reported previously (28). Samples were stored in containers with 4% buffered formaldehyde for fixation, labelled appropriately as right/dorsal, and left/ventral, and subsequently sent to the pathological laboratory, Institute for Pathology, Klinikum Bayreuth.

**Histology**

Routine specimen processing (fixed biopsies are paraffinised after an increasing series of alcohol and xylol, manually orientated by experienced technicians in a perpendicular way, and embedded in a paraffin block; step sections of 4 µm are taken off the block and stained with Haematoxylin & Eosin) was followed by histological analysis performed by the same pathologist (MV). Specimens showing squamous epithelium with a basal cell layer and lamina propria mucosae adequate for histological analysis were classified as adequately sampled from the esophagus. Specimens showing columnar epithelium, defined as foveolar epithelium in combination with basal mucoid glands displaying no or only sparse fundic glands adequate for histological analysis were classified as adequately sampled from the cardia. Ideally both mucosae were present in one biopsy specimen (Fig. 2).

Since each container had been labelled with the respective origin of each sample, specimens could be assigned to the sample-sites for every dog, and the presence or absence of targeted mucosa could be estimated in every dog by histological examination (esophagus and cardia in one biopsy, only esophagus, only cardia, no adequate material). Results were specified on the histology report forms.

Morphometric data from esophageal specimens was analysed digitally using a software program (Analysis®, Olympus Medical Hamburg, Germany). In a subgroup of 43 dogs the thickness of the basal cell layer, the length of stromal papillae, and the total thickness of the epithelium had been measured in micrometers as reported previously (45), and results were noted on the histology report forms.

**Table 1**  
*Histological beurteilbare Proben von Ösophagus und Kardia bei 58 Hunden*

<table>
<thead>
<tr>
<th>Sample site at the gastro-esophageal junction</th>
<th>Dogs with biopsies adequate for histological judging</th>
</tr>
</thead>
<tbody>
<tr>
<td>dorsal esophagus</td>
<td>47 out of 58</td>
</tr>
<tr>
<td>ventral esophagus</td>
<td>46 out of 58</td>
</tr>
<tr>
<td>cardia of the greater curvature</td>
<td>42 out of 58</td>
</tr>
<tr>
<td>cardia of the lesser curvature</td>
<td>47 out of 58</td>
</tr>
</tbody>
</table>

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Statistics

The variables evaluated were: presence of squamous epithelium originating from the dorsal esophagus, presence of squamous epithelium originating from the ventral esophagus, presence of columnar epithelium originating from the cardia of the greater curvature, and presence of columnar epithelium originating from cardia of the lesser curvature, respectively (main variables). Race, sex, age, bodyweight, clinical signs, date of gastroscopy, presence of endoscopic lesions, presence of structural lesions of the esophageal squamous epithelium, and morphology were the data gathered from the clinical examination form and the histology report (influencing variables). The primary outcome was reached, when both esophageal and cardiac mucosa was reported in one biopsy. The Kolmogorov-Smirnov-Test was used to test for normal distribution of the quantitative data. Data of main variables and influencing variables were summarized for the sample and given in absolute and relative numbers. Data of the influencing variables were collected descriptively, and their influences on the main variables and primary outcome were tested by Chi²-Test, followed by Fisher’s exact test, simple regression analysis, and correlation analysis of Pearson, respectively. Morphometric data (given as mean ± standard error) were tested for differences using ANOVA. For statistical procedures the level of significance was p < 0.05. Analysis was supported by the use of a commercially available computer package (WinSTAT® for Excel, R. Fitch Software, Bad Krozingen, Germany).

Results

Clinical findings

Esophagoscopy was performed in 63 dogs over the course of one year, 58 out of which were included in the study, because esophageal biopsies had been obtained. Included dogs belonged to 23 different pure breeds and 22 mixed breeds, with reported body weights ranging between 4.0 kg and 51.0 kg (median 17.0 kg), and ages ranging between 1.0 year and 14.0 years (median 5.0 years). Dogs weighing between 5 and 10 kg comprised 25% of the study population. The age distribution showed two peaks for a range from 1.0 to 2.5, and 5.0 to 7.5 years, respectively (25.9%, and 32.8% of the study population, respectively). Thirty-seven dogs were male (with 18 neutered), and 21 were female (with 16 neutered).

Reported clinical signs were regurgitation (n = 19), dysphagia (n = 2); signs of ptyalism including lip licking, smacking, empty swallowing, and obvious hypersalivation (n = 25); signs of discomfort or pain (n = 28); vomiting (n = 15), and small bowel diarrhoea.
(n = 27). Esophageal clinical signs indicating upper gastrointestinal endoscopy such as regurgitation, dysphagia, ptyalism, and discomfort were reported in 22 out of 58 dogs, and gastrointestinal clinical signs indicating upper gastrointestinal endoscopy such as vomiting, small bowel diarrhoea, and abdominal pain were reported in 36 out of 58 dogs, respectively.

**Esophagoscopy and biopsy**

Identification of the gastro-esophageal junction was reported in all gastroscopies, but the individual colour contrast of the junction varied markedly (▶ Fig. 1). In 55 out of 58 dogs the gastro-esophageal junction was located at the level of the hiatus or little cranial from it. Three out of 58 dogs showed small axial hiatal hernias. In these dogs the gastro-esophageal junction was displaced 2–4 cm cranially and the subsequent gastric folds formed a short tube sliding back and forth during respiration. Course and recognisability of the gastro-esophageal junction were influenced by several factors such as diaphragmatic movements, contractions of the heart, grade of insufflations of the stomach, and the tonus of the lower esophageal sphincter. Identification was impossible if the dog was panting. Dose-dependent respiratory depressive effects of the propofol anaesthesia were beneficial for investigating the gastro-esophageal junction. Lastly, in dogs with reduced sphincter tonus the gastro-esophageal junction presented more clearly than in dogs with a strong tonus. Most often the gastro-esophageal junction was not located at a right angle to the tip of the endoscope, but dorsally and to the right, a little more away from the tip than ventrally and to the left. None of the 58 dogs presented with lesions such as bleedings, strictures, erosive or ulcerative defects.

![Fig. 5](image1.png)  
**Fig. 5** Biopsy specimens of the esophageal squamous epithelium (E) and the stromal papillae of the lamina propria mucosae (PAP). a) Stromal papillae in relation to the epithelium not apparently elongated (HE, x200); b) Stromal papillae in relation to the epithelium markedly elongated: regenerative epithelial alteration; subepithelial fibrosis und lymphocytic infiltrates in the lamina propria (empty arrow): inflammation (HE, x100).

![Abb. 5](image2.png)  
**Abb. 5** Bioplate des ösophagealen Plattenepithels (E) und der Papillen (PAP) der Lamina propria mucosae. a) Höhe der Papillen in Relation zum Epithel unauffällig (HE, x 200); b) Papillen in Relation zum Epithel deutlich verlängert: regenerative Epithelveränderung; Fibrose und lymphozytäre Infiltrate in der Lamina propria (Blockpfeil): Entzündung (HE, x100)

![Fig. 6](image3.png)  
**Fig. 6** Biopsy specimens of the esophageal squamous epithelium (E) with its basal cell layer (BCL). a) Elongated stromal papillae (regenerative epithelial alteration), but thickness of the basal cell layer related to the epithelium not apparently increased; b) thickness of the basal cell layer markedly increased: regenerative epithelial alteration (HE, x200).

![Abb. 6](image4.png)  
**Abb. 6** Bioplate des ösophagealen Plattenepithels (E) mit Basalzellschicht (BCL). a) Verlängerte Stromapapillen (regenerative Epithelveränderung), aber in Relation zum Epithel nicht auffällig verbreiterte Basalzellschicht; b) Basalzellschicht deutlich verbreitert: regenerative Epithelveränderung (HE, x200)
Table 1 shows the incidence of esophageal and cardiac specimens suited for histological evaluation. Biopsies adequately obtained from the gastro-esophageal junction containing squamous epithelium from the esophagus and columnar epithelium from the cardia (Fig. 2), were reported in 45 out of 58 dogs. At the dorsal site, an adequate biopsy was collected from 31 out of 58 dogs. An adequate biopsy was collected from the ventral site in 36 out of 58, and in both sites in 22 out of 58 dogs. No significantly different incidence was found between locations. Breed, sex, or body weight were not significantly different. Incidence of adequately obtained biopsies increased significantly over time (r = 0.22; p < 0.05), the operator’s learning curve is illustrated as a logarithmic function in Fig. 3. After 6–12 months an adequate biopsy was achieved significantly more often (p = 0.03) (29 out of 33 dogs) than during the first 6 months of the study (16 out of 25 dogs).

Histology

All evaluated specimens (Table 1) showed at least adequate parts of esophageal squamous epithelium with the basal cell layer, and lamina propria mucosae. Deeper layers such as the lamina muscularis mucosae or the tela submucosa with mucoid esophageal glands were rarely obtained (Fig. 4).

Biopsies from 14 out of 58 dogs showed histological lesions of the squamous epithelium and the lamina propria mucosae such as fibrosis, inflammation, parakeratosis, elongation of the stromal papillae, and increased thickness of the basal cell layers, compared to dogs without structural abnormalities. The lesions were more often seen ventrally than dorsally (Table 2). The thickness of the basal cell layers of the ventral esophagus was increased in dogs with esophageal clinical signs (mean 16.8 ± 1.7 µm) compared to dogs with gastrointestinal clinical signs (mean 12.8 ± 1.2 µm; p = 0.06). The stromal papillae of the ventral esophagus were significantly (p = 0.03) elongated in dogs with esophageal clinical signs (mean 61.1 ± 11.3 µm) compared to dogs with gastrointestinal clinical signs (mean 37.3 ± 4.4 µm).

Table 2

Results of the morphometric analysis of the esophageal squamous epithelium in endoscopic biopsy specimens obtained from the gastro-esophageal junction of 43 dogs (data in µm, mean ± standard error)

<table>
<thead>
<tr>
<th>Thickness of the Squamous Epithelium</th>
<th>With Presence of Histopathological Lesions (n = 11)</th>
<th>Without Presence of Histopathological Lesions (n = 32)</th>
<th>Significance p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness of the Squamous Epithelium, Dorsal Esophagus</td>
<td>86.8 (± 7.1)</td>
<td>93.6 (± 6.1)</td>
<td>n. s.</td>
</tr>
<tr>
<td>Thickness of the Squamous Epithelium, Ventral Esophagus</td>
<td>120.8 (± 15.2)</td>
<td>96.8 (± 7.4)</td>
<td>n. s.</td>
</tr>
<tr>
<td>Thickness of the Basal Cell Layer, Dorsal Esophagus</td>
<td>16.4 (± 2.2)</td>
<td>12.0 (± 0.8)</td>
<td>0.03</td>
</tr>
<tr>
<td>Thickness of the Basal Cell Layer, Ventral Esophagus</td>
<td>19.4 (± 2.0)</td>
<td>11.9 (± 0.9)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Length of Stromal Papillae, Dorsal Esophagus</td>
<td>54.5 (± 6.3)</td>
<td>34.0 (± 2.7)</td>
<td>0.001</td>
</tr>
<tr>
<td>Length of Stromal Papillae, Ventral Esophagus</td>
<td>70.5 (± 11.6)</td>
<td>34.9 (± 4.0)</td>
<td>0.00095</td>
</tr>
</tbody>
</table>

n. s. = not significant

Discussion

The goal of this study was to answer whether sampling of adequate biopsies from the gastro-esophageal junction of the esophagus is possible during routine upper gastrointestinal endoscopy. It was hypothesized that adequate biopsies can be repeatedly obtained from the canine gastro-esophageal junction, and that histological lesions are identifiable in adequately obtained biopsy specimens.

It is possible to identify the gastro-esophageal junction with a fiberoptic endoscope (27). In some cases details such as the exact course of the junction line, and structures of smaller vessels may be missed. Better results are obtained by application of high resolution video endoscopy (22). Empirically, a well contrasted junction, a decreased tone of the lower esophageal sphincter, moderate respiration, and an adequately inflated stomach may contribute to improved endoscopic identification and evaluation of the gastro-
esophageal junction. As expected, the endoscopically visible gastro-esophageal junction approximates the histological squamo-columnar junction, where esophageal squamous epithelium is abruptly adapted to the foveolar epithelium of the cardia (10, 31). The observed normal variability in the shape of the gastro-esophageal junction may be due to anatomy, i.e. the angled flange of esophagus and stomach, and the gastro-esophageal sphincter's function as a “screw-stretch-closure” (3, 35). Three dogs with short axial hiatal hernias constituted the most obvious endoscopic findings of the study. Marked alterations such as erosive-ulcerative defects or strictures were not observed. Self-healing effects, non-detection of changes, or esophageal health at time of investigation may contribute to the fact, that lesions are not detectable or below the limit of detection during endoscopy. According to the results of the study, minimal endoscopic lesions such as erythema or vascular architecture of the esophageal squamous epithelium cannot be objectively determined by use of fiberoptic endoscopy due to methodical limits and lack of standardization (15, 17, 18). Future investigations, comparing histopathological results from endoscopically obtained biopsies to systematically collected data from digital video endoscopy will help elucidating potential correlations (5, 21, 22, 37).

In this study 77% of biopsies were adequately sampled from the gastro-esophageal junction, exceeding the results of a previous pilot study (40% adequately sampled specimens) (28). Experiences gained during the pilot study may have influenced the learning curve. Other known factors that may impact the outcome of a gut biopsy study include, but are not limited to: the specimen size, experience of the laboratory, and the pathologist's expertise (42–44). The specific anatomic characteristic of a rough epithelium tightly connected with its substratum represents the most important aspect impeding deep specimen sampling in the dog (15, 28, 33). Results of the study suggest that despite increasing practice, biopsy depth hardly varies among individual dogs. Possible advantages of other biopsy forceps systems still remain to be proven in dogs (6, 14, 24).

However, as the study’s results demonstrate, reaching superficial layers seems to be sufficient for the detection of structural alterations of the squamous epithelium and the lamina propria mucosae. Demonstrated histological changes such as cellular infiltrates, subepithelial fibrosis, increased thickness of the basal cell layer, and elongation of the stromal papillae represent inflammatory and regenerative epithelial processes as known from animal reflux models and gastro-esophageal reflux disease in humans (9, 39). Furthermore, regenerative alterations seem to be most distinctive in the ventral esophageal mucosa. As in humans, the reason for asymmetric distribution is unknown (7). The ventral esophageal surface may be more intensively exposed to gastric contents than the dorsal surface because of the orientation of a dog’s body. With the help of the described endoscopic biopsy technique esophageal pathology can be detected. However, only one highly localized area was sampled in this study and no comparisons with other parts of the macroscopically normal esophagus were made in the studied dogs. Also no age-matched control group was evaluated in order to validate the findings. Even though adequate esophageal biopsies seem to be doable, it is yet unknown when sampling the mucosa of the gastro-esophageal junction is indicated and if it is clinically useful. As the study demonstrates, structural lesions are present in a subset of dogs with upper gastrointestinal signs. The clinical relevance of esophageal squamous epithelium lesions is currently unknown and warrants further studies.

Conflict of interest
The authors confirm that they do not have any conflict of interest.

References
14. Gonzalez S, Yu WM, Smith MS, Slack KN, Rotterdam H, Abrams JA, Lightdale CJ. Randomized comparison of 3 different-sized biopsy forceps for...