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Effect of Endoscopic Forceps on the Quality of Duodenal Mucosal Biopsy in Healthy Cats

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Abstract : Based on the results of previous studies, endoscopic biopsy sample's quality has a major impact on its adequacy for histopathology, and that the nature of the biopsy forceps can influence the specimen quality. The present study compared the effects of three different types of endoscopic biopsy forceps and two different operators on sample quality and adequacy for histopathology in three healthy cats. Every biopsy was performed between the major papilla and caudal duodenal flexure, and each operator performed five biopsies with each type of forceps on each cat, for a total of 90 biopsies. One pathologist evaluated the quality and adequacy of the obtained samples. Biopsies performed with large-cup forceps provided heavier and longer samples than the standard round forceps. With the same size forceps, the presence of alligator teeth had no effect on sample quality or adequacy for histopathological examination and assessment. Based on the results of the present study, although the standard round forceps could be used to obtain adequate samples for histopathology, large-cup forceps such as the standard oval and alligator jaw type have the advantage of obtaining high quality endoscopic samples.

Key words **:** endoscopic biopsy, biopsy forceps, cat.

Introduction

Small intestinal endoscopy is indicated for chronic small bowel diarrhea, ill-defined vomiting disorder, recurrent abdominal pain, weight loss of unknown origin, signs of gastrointestinal bleeding, and nonresponse to empirical treatment of these clinical symptoms in dogs and cats (15). Although endoscopy can be performed with a therapeutic intent, including foreign body retrieval, polyp removal, stricture dilation or feeding tube placement, the main reason of performing endoscopy is to obtain biopsies for histopathological investigation (16). The quality of endoscopically obtained duodenal samples, in particular, is important, owing to several reasons (3,12,19-21). However, obtaining tissue sample specimens can be challenging, as evidenced by the various techniques that have been reported for endoscopic biopsy (7,13). Moreover, the overall quality of a biopsy specimen has a major impact on its adequacy for histopathological examination and assessment, and also that the nature of the endoscopic biopsy forceps such as size, shape, usage, and presence or absence of fenestrations, needle, and alligator teeth of forceps can influence the specimen quality (2,6,9). Therefore, the quality of tissue specimens obtained endoscopically can vary widely, and some samples can be inadequate for histopathologic diagnosis (17). In human medicine, prior studies have shown that in general "larger is better", and that alligator-style cups provide better samples than oval cups of the same size (2,5,14,18). In veterinary medicine, a recent study suggested that large-capacity forceps are superior, providing higher quality duodenal samples for histopathology in healthy dogs (8). However, no data exist regarding the impact of the type of biopsy forceps on endoscopic biopsy sample quality in cats. Indeed, investigations of endoscopic biopsy forceps applied to humans and dogs might not be relevant to feline patients, given the differences in patient size and organ anatomy. The purpose of this study was to evaluate and compare the quality and adequacy of duodenal samples in healthy cats obtained with different types of forceps designed for gastrointestinal endoscopy for histopathological assessment, and to determine if there is any variation in specimen quality associated with different endoscopists.

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Materials and Methods

Animal preparation

This pathologist-blinded study employed three reusable pinch forceps to collect mucosal biopsy samples from the duodenum via upper gastrointestinal endoscopy in three domestic short hair cats. The cats were all clinically healthy, spayed females with no gastrointestinal symptoms (vomit,

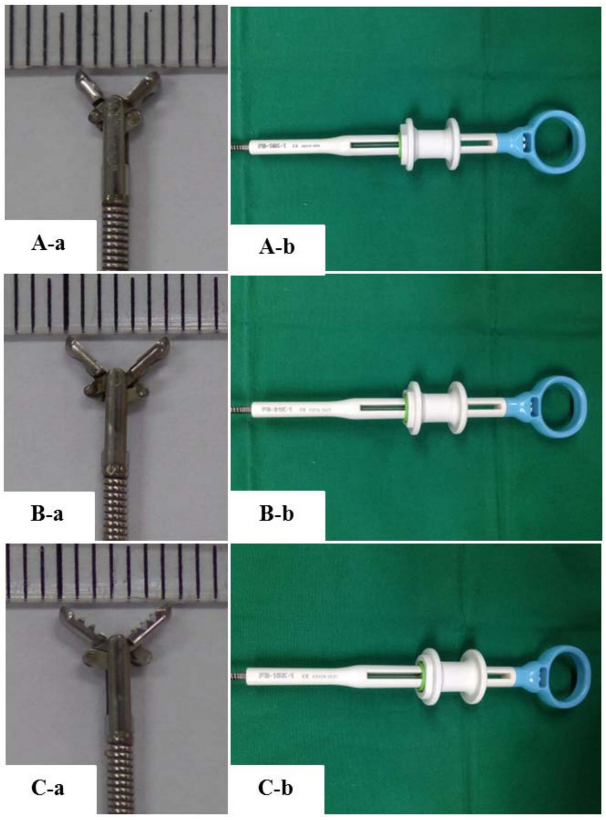

Fig 1. Flexible endoscopic biopsy forceps. A: FB-19K-1 (standard round type) approximately 4.5-mm opened jaw with a fenestrated cup (A-a) and handle piece (A-b). B: FB-21K-1 (standard oval type) approximately 6.0-mm opened jaw with a fenestrated cup (B-a) and handle piece (B-b). C: FB-15K-1 (alligator jaw type) approximately 6.0-mm opened jaw with a non-fenestrated cup (C-a) and handle piece (C-b).

diarrhea, anorexia, or weight loss), normal blood work results (complete blood count [CBC] and serum biochemistry), and no remarkable findings on medical diagnostic images (radiography and ultrasonography). The body weight of the cats varied between 4.0 kg and 4.9 kg (mean weight 4.5 kg). All cats were treated in accordance with the guidelines approved by the Institutional Animal Care and Use Committees (IACUC) of Gyeongsang National University.

Biopsy forceps

Three different reusable forceps were used in this study. The standard shape forceps (FB-19K-1, Olympus, Japan) (Fig 1A) had an approximately 4.5 mm opened jaw with a fenestrated cup (Fig 1A-a); the standard oval forceps (FB-21K-1, Olympus, Japan) (Fig 1B) had an approximately 6.0-mm opened jaw with a fenestrated cup (Fig 1B-a); and the forceps with alligator jaws (FB-15K-1, Olympus, Japan) (Fig 1C) had the same opened cup size as the standard oval forceps (Fig 1C-a). All forceps used in this study were Olympus pinch biopsy forceps with 1.8-mm diameters (Fig 1), which is a smaller size than the primary forceps used in the previous studies.

Endoscopic biopsy procedure

Gastroduodenal endoscopy was performed using a flexible video endoscope (GIF XP 150N Flexible Video Endoscope; working length: 110 cm, outer diameter: 5.5 mm, working channel diameter: 2.0 mm, Olympus, Japan) with a CV-150 processor. Two different operators performed the endoscopic biopsy procedures in this study. Samples were obtained from normal-appearing duodenal mucosa between the major papilla and caudal duodenal flexure. Operators collected all duodenal mucosal specimens using the "turn-and-suction" technique (10).

The forceps cup is opened after passage through the accessory channel of the endoscope, and the forceps is withdrawn against the tip of the endoscope. Then, the endoscope tip and forceps are directed and turned towered the mucosa and suction is employed as the forceps is gently applied and closed. Each duodenal tissue specimen was retrieved from the forceps and unfolded using a 25-gauge needle. The unfolding process was performed very carefully to prevent stretching or tearing the samples. All biopsy samples were placed into individual vials containing 10% formaldehyde within a few seconds.

Tissue samples were taken by two operators with similar experience and technique. Each operator performed five duodenal biopsies with each type of forceps, for each cat, leading to a total of 90 biopsies.

Weighing of specimen

Before the endoscopic biopsy procedure, vials were filled

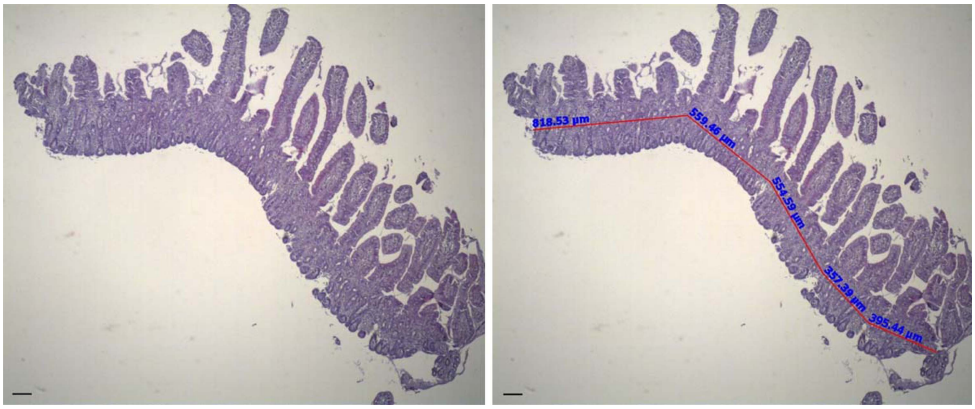

Fig 2. Histological measurement of a biopsy sample length from the feline duodenum. The length is measured by using ZEISS AxioVision software.

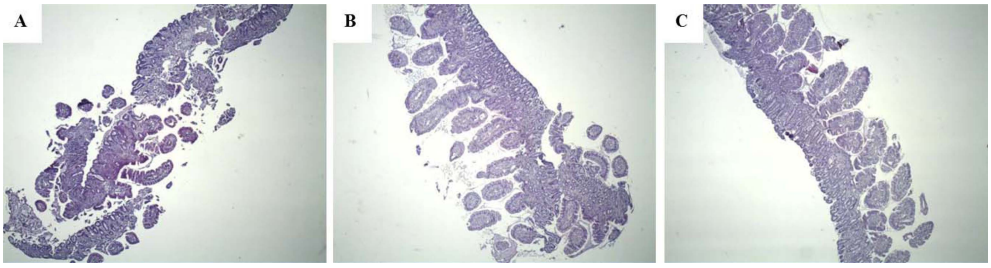

Fig 3. Histological examination of biopsy samples from the feline duodenum illustrating the crush artifact score. Hematoxylin and eosin (H&E) stain. (A): Maximum artifact. (B): Intermediate artifact. (C): Minimum artifact.

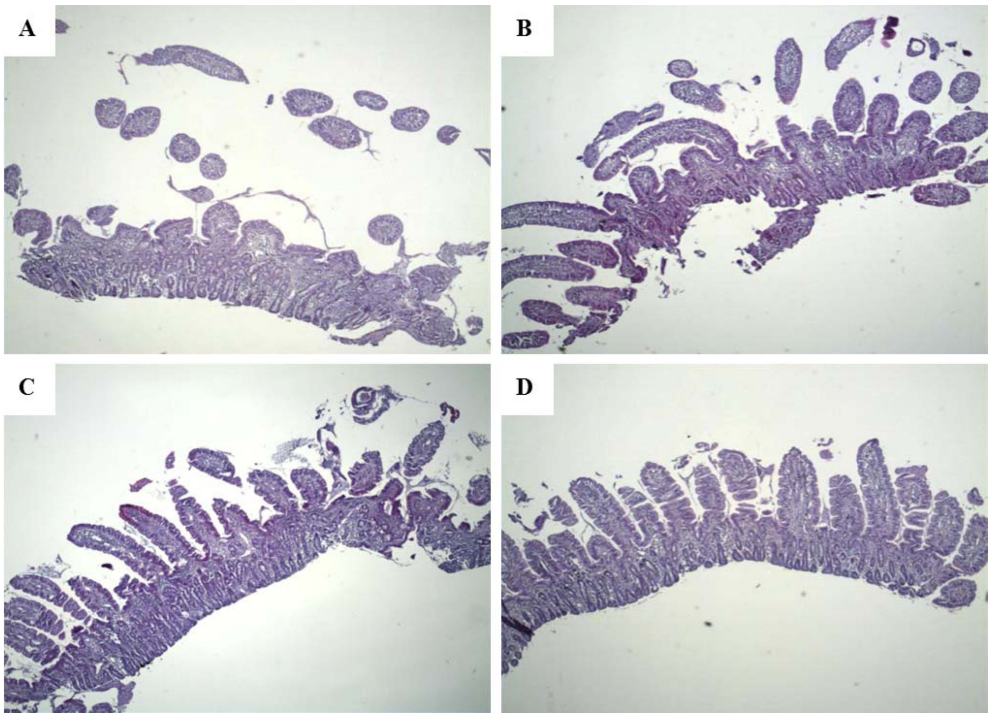

Fig 4. Histological examination of biopsy samples from the feline duodenum. Representative samples of each histopathological adequacy assessment score. Hematoxylin and eosin (H&E) stain. Magnification × 40. (A): An "inadequate" biopsy sample lacks the full thickness of the villus and subvillus. (B): A "marginal" biopsy sample has at least 1 villus and the subvillus. (C): An "adequate" biopsy sample has at least 3 villi and the subvillus. (D): A "superior" biopsy sample has at least 7 villi and the subvillus.

with approximately 1 mL 10% formaldehyde and capped. All of the vials were weighed on a digital scale (Adventurer, Ohaus) to the nearest 0.1 mg 6 h before the biopsy. A biopsy sample was placed in each vial, and every vial was weighed again with same scale within 3 h. Subsequently, the weight of each specimen was calculated by subtraction.

Biopsy scoring

After measuring the weight of the sample, a total of 90 vials filled with 10% formaldehyde were referred to the Pathologic Diagnosis Service of the Veterinary College of Gyeongsang National University. The tissues were embedded in paraffin and then sectioned serially 5 μm thick, and stained with hematoxylin and eosin (H&E) according to manufacturer instructions. Each slide was evaluated and graded by one pathologist for the following characteristics: length, depth, crush artifacts, and adequacy for histopathological examination (8).

Length (in millimeters) (Fig 2)

Sample length was measured using ZEISS AxioVision software.

Depth (Scored from 1 to 4) (8)

Each tissue sample was scored as follows: 1 = Very superficial; 2 = Mucosa only; 3 = Muscularis mucosa in the section; 4 = Submucosa in the section

Crush artifact (Scored from 1 to 3) (8)

Each tissue sample was scored as follows: 1 = Maximum artifact (Fig 3A); 2 = Intermediate artifact (Fig 3B); 3 = Minimum artifact (Fig 3C)

Adequacy for histopathological examination (Scored from 1 to 4; 1 = Inadequate, 2 = Marginal, 3 = Adequate, 4 = Superior) (8) The definition of each grade is as follows:

"Inadequate sample" refers to specimens that had only villi or subvillus lamina propria, but not both (Fig 4A)

Table 1. Effect of endoscopists on biopsy specimen weight, length, depth, crush artifact, and adequacy for histopathology

	Standard type round shape (FB-19K-1)		Standard type oval shape (FB-21K-1)		Alligator jaw type (FB-15K-1)	
	Operator 1	Operator 2	Operator 1	Operator 2	Operator 1	Operator 2
Weight (Mean in mg ± SD)	0.0017 ± 0.0007	0.0017 ± 0.006	0.0026 ± 0.0014	0.0028 ± 0.0012	0.0025 ± 0.0082	0.0020 ± 0.0072
Length (Mean in μm ± SD)	2534.25 ± 526.50*	1846.27 ± 785.71	3009.57 ± 650.46	2656.04 ± 771.28	3038.33 ± 936.40	2809.03 ± 818.28
Depth (Mean in score ± SD)	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	1.87 ± 0.35
Crush Artifact (Mean in score ± SD)	2.73 ± 0.59	2.40 ± 0.74	2.73 ± 0.46	2.53 ± 0.52	2.67 ± 0.62	2.33 ± 0.62
Adequacy for histopathology (Mean in score ± SD)	2.40 ± 0.63	2.46 ± 0.99	2.60 ± 0.99	2.63 ± 0.85	2.87 ± 0.92	2.73 ± 1.10

Each value represents the mean ± SD of 90 samples. *Significant differences are observed in sample length between operators for samples obtained with the standard round forceps ($p < 0.05$).

Table 2. Effect of biopsy forceps features on biopsy specimen weight, length, depth, crush artifact, and adequacy for histopathology

Shape	Size (mm)	Weight * (Mean in mg ± SD)	Length * (Mean in μm ± SD)	Depth (Mean in score ± SD)	Crush Artifact (Mean in score ± SD)	Adequacy for histopathology (Mean in score ± SD)
Standard type round shape (FB-19K-1)	Working channel 2.0 Jaw open 4.5	0.0017 ± 0.0066[a]	2190.26 ± 744.48[a]	2.00 ± 0.000[a]	2.57 ± 0.679[a]	2.43 ± 0.817[a]
Standard type oval shape (FB-21K-1)	Working channel 2.0 Jaw open 6.0	0.0026 ± 0.0013[b]	2832.80 ± 723.71[b]	2.00 ± 0.000[a]	2.63 ± 0.490[a]	2.63 ± 0.850[a]
Alligator jaw type (FB-15K-1)	Working channel 2.0 Jaw open 6.0	0.0022 ± 0.0010[a,b]	2923.68 ± 871.86[b]	1.93 ± 0.254[a]	2.50 ± 0.630[a]	2.80 ± 0.997[a]

Each value represents the mean ± SD of 90 samples. For each parameter (weight, length, depth, crush artifact, and adequacy for histopathology), values followed by the same superscript letter (a, b, or c) are not significantly different ($p > 0.05$). *Significant differences are observed in sample weight and length ($p < 0.05$).

"Marginal sample" refers to specimens that had at least 1 villus plus subvillus lamina propria (Fig 4B)

"Adequate sample" refers to specimens that had at least 3 villi plus subvillus lamina propria (Fig 4C)

"Superior sample" refers to specimens that had at least 7 villi plus subvillus lamina propria (Fig 4D)

The evaluating pathologist was blinded to all information, including the identity of the endoscopist, type of biopsy forceps used, and identity of the animal.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) program, version 12.0. One-Way ANOVA and Mann-Whitney U tests were used to compare the sample quality (including weight, length, depth, and crush artifact) and histopathological adequacy, respectively, between the types of endoscopic forceps and the different operators. Post-hoc pairwise comparisons were examined using Tukey's test as appropriate. In all analyses, a value of $p < 0.05$ was considered statistically significant.

Results

There was a statistically significant difference in sample length between the two operators for the specimens that were obtained using the standard round forceps ($p = 0.007$) (Table 1 and Fig 6). There were no significant differences in weight ($p = 0.901$), depth ($p = 1.000$), crush artifact ($p = 0.141$), or adequacy for histopathology ($p = 0.789$) (Table 1).

The samples obtained with the standard oval cup forceps were significantly heavier than those obtained with the standard round cup forceps ($p = 0.000$), but there were no statistically significant differences between the alligator jaw type and the standard round ($p = 0.072$) or standard oval ($p = 0.199$) (Table 2).

As shown in Table 2, the length of the tissue obtained with the standard round forceps was significantly shorter than that obtained with the standard oval forceps ($p = 0.006$) and alligator jaw-type forceps ($p = 0.001$). There was no significant difference between the standard oval and alligator jaw-type forceps ($p = 0.895$).

In all samples except the two obtained using the alligator-type forceps, the depth of tissue was scored as 2. The mean depth score was not statistically different among the biopsy forceps ($p = 0.188$) (Table 2).

There was no statistically significant difference in crush artifact across the forceps types ($p = 0.671$) (Table 2).

Among the forceps used in this study, there was no significant difference between them in their adequacy for histological examination and assessment ($p = 0.254$) (Table 2).

Discussion

The results of this study demonstrate the superiority of large-cup forceps. In particular, the size of the biopsy forceps cup has a significant effect on the weight and length of feline duodenal endoscopic biopsy samples, regardless of the operator. For sample weight, biopsies performed with the standard oval forceps were significantly heavier than those performed with the standard round forceps. For sample length, biopsies obtained with the standard oval and alligator type were significantly longer than biopsies obtained with the standard round type. This result was similar to those reported by previous studies in humans and dogs, wherein "larger is better" was asserted for sample weight and length. Based on these results, although there was no significant difference in sample depth, crush artifact, and adequacy for histopathology among the three forceps, we recommend the use of biopsy forceps with a large size cup when obtaining duodenal mucosal samples in cats.

As for other comparisons, previous studies did not demonstrate meaningful differences in sample quality across forceps styles (1,2,5). Alligator-tooth forceps can obtain deeper specimens (22), however, in many studies the presence of alligator-tooth forceps did not have a significant influence on the sample quality and adequacy for histopathological examination and assessment (2,4,5,14,22). In the present study, alligator jaw forceps significantly impacted only sample length, not sample depth, compared to standard round forceps. When compared with the same forceps cup size, however, there was no meaningful difference in sample quality and adequacy between alligator jaw and oval forceps. In addition, fenestration was present in the cup of the standard oval forceps but not the cup of the alligator jaw forceps. Therefore, additional comparisons (fenestrated alligator jaw type versus closed alligator jaw, closed standard oval versus fenestrated standard oval) are needed to evaluate whether fenestration of the cup influences sample quality.

Unlike the difference in weight and length, there was no significant impact of forceps type on sample depth, crush artifact, or adequacy for histopathology. In comparison to our study, large-capacity forceps (2.4 mm, 3.2 mm diameter) provided significantly better samples with regard to adequacy for histopathology than other small forceps (1.8 mm diameter) in humans (5,6,11) and dogs (8). We were surprised that there were no differences in the adequacy for histopathology and crush artifact when comparing a small size cup to a large size cup. Endoscopic specimens from small cup forceps generally obtain small tissue samples (20), and smaller specimens tend to fragment more easily and it is technically more difficult to achieve the correct sample orientation with a smaller amount of tissue (5). In general, especially compared with larger dogs, cats have a relatively thinner intestinal mucosa, which could make it easier for endoscopists to procure mucosal samples, even when using endoscopes with smaller diameter biopsy channels (21). Based on this factor, small size forceps, such as the 1.8-mm standard round type, can provide adequate tissue samples for histopathology with a low crush artifact from the feline duodenum.

Interestingly, regardless of the forceps type, none of the specimens obtained in our study contained the muscularis mucosa layer, even though they were evaluated as having adequate or superior sample score. Similar studies in humans and dogs have found that samples obtained with large-capacity forceps had no difference in depth compared to smaller forceps, despite significant differences in length and weight (2,4,8). The size and depth of the specimen are dependent on the forceps diameter size and pressure applied to the forceps during the biopsy procedure (5). Specimens obtained with the 2.4 mm diameter forceps were significantly larger and deeper than those obtained with the 'pediatric' 1.8 mm diameter forceps (5). All forceps in this study were pediatric forceps that were insufficient for capturing the deeper layer and applying appropriate pressure at the mucosa, though the three forceps had different styles and cup sizes. It is recognized that these pediatric forceps are limited in their ability to obtain high quality samples with regard to depth.

However, the length and weight of the samples are more closely related to histological adequacy than is sample depth (2,4). The superior weight and length of the samples, even without an improved depth, could be sufficient to improve adequacy for histology and the ability to provide an appropriate diagnosis (2,4). Similar to previous studies, the results of the present study revealed that higher length and artifact scores were associated with more adequate and superior samples, despite no relationship with depth. We thought that adequacy for histology was evaluated by the number of villi in the sample, which is affected by sample length. Although we did not identify a direct relationship between forceps type and adequacy for histology in the present study, the large-cup forceps may influence adequacy indirectly by its positive impact on sample quality. Thus, large forceps have an advantage for use in endoscopic duodenal biopsy in cats.

There are some limitations in this study. First, the number and variety of biopsy forceps were fewer than those reported in previous studies (1,2,5,6,8,11,18,23). For a more meaningful comparison, it will be necessary to evaluate more varied forceps types (large diameter, those with a spike, disposable, different manufacturers) to determine the impact of the type of forceps on sample quality. Second, our study was limited practically by the sample handling procedure in the sample submission technique, because we did not rule out variability during the unfolding process by using a needle. Third, all specimens were taken from healthy duodenal mucosa, so these results may not be applicable in other circumstances because upper gastrointestinal diseases likely result in a more fragile and friable mucosa. Finally, the endoscopic samples taken from other sites of the GI tract such as the stomach, jejunum, ileum, and colon of cats might not show the same

results seen with the duodenal mucosa.

The present study showed the effects of biopsy forceps type on duodenal mucosal sample quality in healthy cats. Based on the present study, there was no significant difference in the adequacy for histopathology and assessment among the standard round (FB-19K-1), standard oval (FB-21K-1), and alligator jaw (FB-15K-1) forceps. However, the standard oval and alligator jaw forceps could provide better sample quality in the length and/or weight parameters. Therefore, we recommend the use of large-size cup forceps for duodenal mucosal biopsies in cats to obtain better quality specimens. In addition, further studies are needed to evaluate more forceps types and forceps effects on samples from cats with gastrointestinal disease.

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고양이 십이지장 점막 생검 시 내시경 생검 겸자가 조직의 질에 미치는 영향에 관한 연구

원진희 · 홍일화 · 장효미 · 엄나영 · 지초희 · 정해원 · 강병택* · 정동욱 · 정동인**[1]**

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요 약 : 본 연구는 고양이 십이지장에서 내시경적 조직 생검 시 사용된 다양한 겸자의 특징에 따른 조직의 질과 조직병리학적 적합성에 대해 평가하였다. 이러한 내시경용 생검 겸자에 따른 조직의 질과 적합성에 대한 연구는 인의에서 다수 보고되었으며 최근 개에서의 연구가 보고되었으나 고양이에서의 연구는 진행되지 않았다. 따라서 본 연구에서는 기존의 연구 결과와 고양이에서의 결과를 비교하여 그 차이를 확인하고자 하였다. 총 3마리의 건강한 고양이의 십이지장에서 3가지 종류의 생검 겸자를 사용하여 두 명의 실험자가 각각의 겸자 당 5 개씩의 시료를 채취하여 총 90개의 십이지장 점막 조직을 획득하였다. 획득한 조직은 각각 무게와 길이, 깊이, 부서진 인공구조, 조직병리학적 적합성에 대해 평가하였다. 내시경 생검용 겸자에 의한 조직의 질은 컵이 큰 겸자의 경우 더 무겁고 긴 조직을 얻는데 효과적인 결과를 얻었으며 이는 기존의 사람과 개에서의 연구와 비슷하다. 그러나 조직의 깊이와 부서진 인공구조, 특히 조직병리학적 적합성에 있어서는 겸자의 컵 크기나 모양에 따른 차이가 나타나지 않았으며 이는 기존의 연구와 다른 점이다. 하지만 조직학적 적합성이 있다고 평가된 조직들은 길이가 길고 인공구조가 적게 나타났으며 이러한 조직의 질은 겸자의 종류에 따른 차이를 보였다. 따라서 겸자의 종류는 조직의 질에 영향을 미치며 이는 간접적으로 조직학적 적합성과 관련이 있을 것으로 생각된다. 이를 바탕으로 고양이의 내시경적 생검 시, 비록 작은 컵의 겸자로도 조직병리학적으로 적합한 조직을 얻을 수 있지만 더 나은 질의 조직을 얻기 위해서 큰 컵의 겸자를 적용하는 것이 효과적인 것으로 생각된다.

주요어 : 내시경적 조직 생검, 생검 겸자, 고양이