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Evaluating Quality and Adequacy of Gastrointestinal Samples Collected using Reusable or Disposable Forceps

J.A. Cartwright, T.L. Hill, S. Smith, and D. Shaw

Background: Sample quality of gastrointestinal endoscopic biopsies is of paramount importance for accurate histological diagnosis. Many veterinary practices use reusable forceps as a result of perceived decreased cost. With reusable forceps, it remains unknown whether sample quality declines with repeated use and becomes inferior to single-use forceps and is therefore more or less cost effective than single-use forceps.

Hypothesis/Objectives: The study hypothesis was that reusable forceps sample quality would deteriorate after repeated use as compared to single-use forceps.

Animals: Sixty-five dogs undergoing gastrointestinal endoscopy for diagnostic investigations at the Hospital for Small Animals, Edinburgh University.

Method: A prospective, pathologist-blinded study comparing single-use and reusable alligator standard cup biopsy forceps (Olympus 2.0 mm 1550 mm) with 5 randomized reusable forceps. Sample quality (stomach, duodenum, ileum, and colon) was assessed by a single pathologist using the WSAVA guidelines.

Results: There was no difference in the adequacy, depth, villi number, or crush artifact in the 4 intestinal areas between forceps type with at least 10, and up to 15, repeated uses of the reusable forceps.

Conclusions and clinical importance: This study demonstrates that reusable cup biopsy forceps provide equivalent biopsy quality after repeated uses to single-use forceps and are cost effective at 10-case use.

Key words: Dogs; Endoscopy; Enteropathy; Inflammatory Bowel Disease.

Endoscopic biopsy of gastrointestinal tissue is commonly performed in referral veterinary hospitals and primary care practices to obtain diagnostic information in companion animals presenting with chronic gastrointestinal signs. A histological assessment is required to diagnose types of chronic enteropathies, including lymphocytic-plasmacytic enteritis, eosinophilic enteritis, granulomatous enteritis, lymphangiectasia, and neoplasia, eg, gastrointestinal lymphoma.[1,2] Endoscopic biopsies have reduced morbidity as compared with those identified with surgical full-thickness biopsies.[3] Endoscopic biopsies are more superficial than surgical biopsies, so sample quality is paramount to allow a histopathological diagnosis.[4] The World Small Animal Veterinary Association has published assessment criteria for canine gastrointestinal biopsy samples, which provides guidelines for biopsy quality assessment and histopathologic findings.[5] Sample quality is especially important when differentiating inflammatory bowel disease from lymphoma as these two diseases can be difficult to differentiate.[6]

Abbreviations:

IBD	inflammatory bowel disease
WSAVA	World Small Animal Veterinary Association

In people, the cost effectiveness and relative risk: benefit of reusable versus single-use forceps is uncertain, with some publications indicating that single-use forceps provide superior sample quality.[7–9] Although several different biopsy forceps are available to the veterinary profession, many small animal practices and referral hospitals employ reusable biopsy forceps. It is currently unknown whether sample quality degenerates with repeated use of a reusable biopsy forceps in multiple dogs compared with single-use forceps (use/dog) and the time period over which this occurs. Declining function of reusable forceps is illustrated in people after 24 single uses, although the method of sampling is not comparable to veterinary gastrointestinal endoscopy.[10] At the author's institution, reusable forceps become more cost effective as compared to single-use forceps after 10 dogs. The aim of this study was to compare the adequacy and quality of reusable and single-use gastrointestinal biopsy samples for histological examination and to establish a numerical limit where reusable alligator standard cup biopsy forceps show decline in sample quality compared to single-use forceps beyond the point of cost effectiveness (10 dogs).

Materials and Methods

This study was approved by the University of Edinburgh Veterinary Ethical Review Committee. Endoscopic biopsy samples were collected from consecutive dogs undergoing gastrointestinal

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Table 1. Quality Scoring of Each Sample for Stomach, Duodenum, and Ileum.

Measured trait	Score description	Score
Depth	Very superficial	1
	Mucosa only	2
	Muscularis mucosae present in the section	3
	Submucosa present in the section	4
Crush artifact	Minimum, affecting <5% of the section*	1
	Intermediate, affecting up to 30% of the section*	2
	Maximum, affecting >30% of the section*	3
Number of whole Villi present.	Numerical	NA
Gastric slides adequacy	Inadequate: only superficial mucosa and epithelium, or deep mucosa, but not both	1
	Marginal: epithelium and mucosa, but did not clearly have full-thickness mucosa	2
	Adequate: full-thickness mucosa, whether or not it included muscularis mucosae	3
Duodenal slides adequacy	Inadequate: Only villi or subvillus lamina propria, but not both	1
	Marginal: At least one villus plus subvillus lamina propria, but did not clearly have full thickness of the subvillus lamina propria extending to the muscularis mucosae	2
	Adequate: At least three villi and subvillus lamina propria that extended to the mucosa-muscularis mucosae border	3
	Superior: At least seven villi with subvillus lamina propria that extended to the mucosa-muscularis mucosae border, whether or not it included muscularis mucosae	4
Ileal slides adequacy	Inadequate: Only villi or subvillus lamina propria, but not both	1
	Marginal: At least one villus plus subvillus lamina propria, but did not clearly have full thickness of the subvillus lamina propria extending to the muscularis mucosae	2
	Adequate: At least three villi and subvillus lamina propria that extended to the mucosa-muscularis mucosae border	3
	Superior: At least seven villi with subvillus lamina propria that extended to the mucosa-muscularis mucosae border, whether or not it included muscularis mucosae	4

*Adaptation by pathologist SS.

endoscopy for investigation of gastrointestinal disease at the Royal (Dick) School of Veterinary Studies, Hospital for Small Animals in a prospective, pathologist-blinded study over a 1 year period from January 2014 to January 2015. Two types of forceps were compared, single use and reusable. Each reusable forceps (A–E) was new at the start of the study. One of the reusable forceps (A–E) was used in each dog along with a new single-use biopsy forceps.[a] The reusable forceps to be used in each dog were determined by random number generation and the forceps were reprocessed in accordance with manufacturer's guidelines between each endoscopy. Briefly, this included manual macroscopic cleaning, ultrasonic cleaning, and a final wash in the scope washer with Gigasept. The endoscopist and endoscopy technician performing and assisting with each procedure were recorded. Samples were collected with both reusable and single-use forceps in each dog until at least economical equivalence was reached with each reusable forceps (A–E) at 10 uses (10 dogs). The order of forceps use (A–E) was randomized, so to ensure all forceps were used at least 10 times, some forceps were used beyond this value; 1 forceps was used for 10 dogs, 2 for 12, 1 for 14, and 1 for 15.

Collection of Biopsy Samples

Residents or senior faculty with varying endoscopy experience performed gastrointestinal endoscopy with a flexible video gastroscope.[b] Four biopsy samples were taken with each forceps type from each segment sampled (stomach, duodenum, ileum, colon). The managing case clinician determined the segments of the gastrointestinal tract sampled; at clinician discretion, each forceps obtained 4–16 samples (1–4 gastrointestinal tract segments) per dog. The routine order for endoscopic sampling was duodenum, stomach, ileum, and colon. Samples were obtained alternately with single-use and reusable forceps. The forceps type used first for each endoscopy was also randomized by random number generation. Forceps malfunctions were recorded.

Preparation, Storage, and Transport of Samples

Samples were gently transferred from the forceps with a 25 g hypodermic needle onto cellulose nitrate paper presoaked in formalin along the same plane with the mucosa oriented upwards to allow

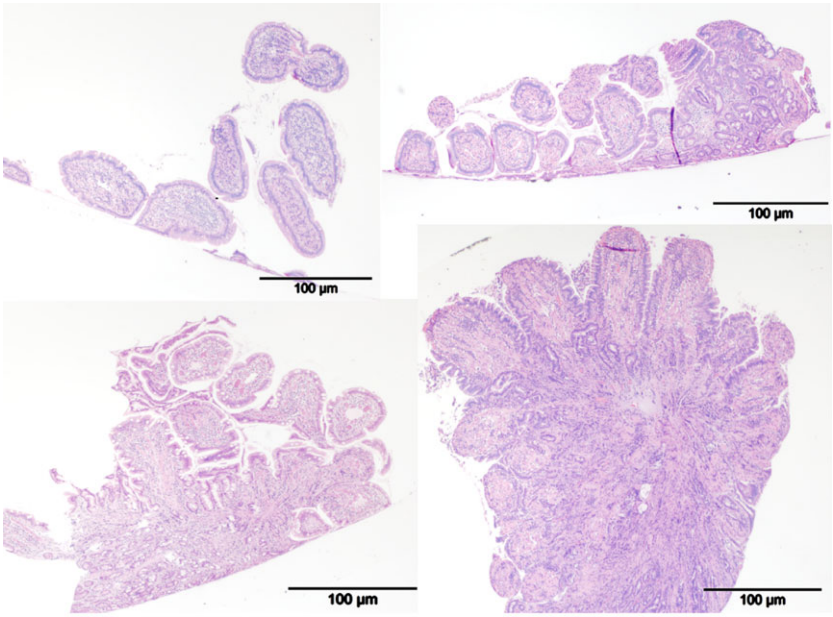

Fig 1. Example biopsy sections with adequacy scores 1–4 (all pictures are of the duodenum). Top left to right score 1 and score 2. Bottom left to right score 3, and score 4.

for processing of all samples simultaneously. The filter papers with samples were placed in a ratio of approximately 1:50, 10% buffered formalin, and tissue processing was standardized as previously described.[11] Briefly, the samples were retained on the cellulose nitrate filter paper for processing to allow standardized orientation when sectioning. In addition to previously described processing, a piece of specialized paper was placed on the samples to prevent detachment.

Sample Analysis

A certified pathologist (SS) was blinded to forceps type for assessment of quality. Samples were analyzed for quality with the WSAVA Gastrointestinal Standardization guidelines[12–14] (Table 1, Fig 1). In brief, the depth and overall quality were scored from 1 to 4, with 1 being inadequate and 4 being superior. Crush artifact was scored as 1 (minimum) to 3 (maximum) and whole villi number was recorded for the duodenum and ileum. The pathologist (SS) developed criteria for colonic assessment of sample quality using the templates set for stomach, duodenum, and ileum in previous studies[12–14] and those previously described in humans[15] (Table 2). Histological changes were classified as normal, mild, moderate, or marked inflammation and the presence of neoplastic lesions was documented.

Statistical Analysis

The median score for depth, adequacy, number of entire villi, and crush artifact from each gastrointestinal segment and forceps type was calculated. To examine for baseline differences between a new reusable and single-use forceps, a Wilcoxon rank sum test compared the median scores of the first use of each reusable forceps with the median scores of the single-use forceps.

When assessing the biopsy quality degradation over time, statistical analysis was completed once all reusable forceps had been used in 10 dogs. The median score for the reusable forceps was subtracted from the median score for the single-use forceps from each dog and gastrointestinal segment for each of the 4 quality assessments (depth, adequacy, number of entire villi, and crush artifact) to generate a difference of median scores. Linear mixed-effect models of difference of median scores for each section were used for longitudinal statistical analysis of the reusable forceps, where reusable forceps (A–E) were entered as the random effect to take account of the repeated measures. The endoscopist, endoscopy technician, and pathology score were entered into separate models as fixed effects and the number of uses as a covariate for each area of gastrointestinal tract (stomach, duodenum, ileum, and colon). Standard linear regressions of adequacy, depth, crush, and villi median values were analyzed with the pathology score as covariate for single-use forceps only for each area of gastrointestinal tract (stomach, duodenum, ileum, and colon).

Table 2. Quality Scoring of Each Sample for Colon.

Colon slides adequacy	Inadequate: Only mucosal epithelium or superficial lamina propria, but not both	1
	Marginal: ≥10 crypt, but does not extend to the muscularis mucosa	2
	Adequate: ≥10 crypts and full-thickness mucosa with intact lining epithelium and muscularis mucosae	3
	Superior: ≥20 crypts and full-thickness mucosa with intact lining epithelium and muscularis mucosae	4

The 6 endoscopists that performed ≥4 endoscopies and 5 endoscopy technicians that assisted ≥5 endoscopies were analyzed with a 1-way Kruskal-Wallis test and a nonparametric posthoc test: Dwass-Steel-Critchlow-Fligner. Analysis was carried out in R (v3.1.2, (c) 2014 The R Foundation for Statistical Computing) with the 'nlme' package (v 3.1-120). Significance was set at $p < .05$.

Results

Sample Population

Sixty-five consecutive dogs representing 28 breeds were included for analysis. Median age was 5 ½ years (range 3 months–14 years). Clinical signs included

vomiting, diarrhea, anorexia, weight loss, constipation, hematemesis, melena, and hematochezia. Three dogs had histologically unremarkable samples, 34 had mild inflammatory changes, 24 had moderate inflammation, 2 had marked inflammatory changes, and 2 were diagnosed with adenocarcinoma.

Five senior clinicians and 6 residents performed endoscopies with 1 clinician and 2 residents performing the majority of endoscopies (13, 12, and 17, respectively). Ten different endoscopy technicians assisted in sample processing. Each forceps type obtained 4 samples from each gastrointestinal segment sampled (stomach, duodenum, ileum, colon) dependent on clinical requirement for the dog. Each reusable forceps was used in 10–15 dogs and the total number of samples obtained per reusable forceps was between 124 and 172.

Biopsy Sample Quality

Biopsy samples were of equivalent quality at baseline for each reusable forceps when compared to single-use forceps. Similarly, sample quality, assessed by adequacy, depth, crush artifact, and number of villi did not decline over time after at least 10 and up to 15 dogs per reusable forceps (Figs 2 and S1–S3). Degree of inflammation (normal, mild, moderate, or marked) did not affect sample quality, either for single-use forceps or over time with reusable forceps.

There were 6 endoscopists who had biopsied at least 4 dogs (range 4–17). Median stomach depth scores were 0.5 different between two endoscopists with single-use forceps ($p = .032$). Two endoscopists produced higher median duodenal depth and adequacy scores with single-use forceps as compared to reusable forceps ($p = .027$ and .038, respectively). The remaining endoscopists provided equivalent samples based on forceps type.

Of the 5 endoscopy technicians assisting with more than 4 procedures, 1 technician was associated with higher median gastric depth scores with the single-use versus reusable forceps ($p = .004$). This technician had a high percentage of samples at depth score 3 for the 5 endoscopies she assisted with. The remaining technicians provided equivalent samples based on forceps type.

Three malfunctions were recorded among the 5 reusable forceps for the first 10–15 dogs; 1 malfunction was

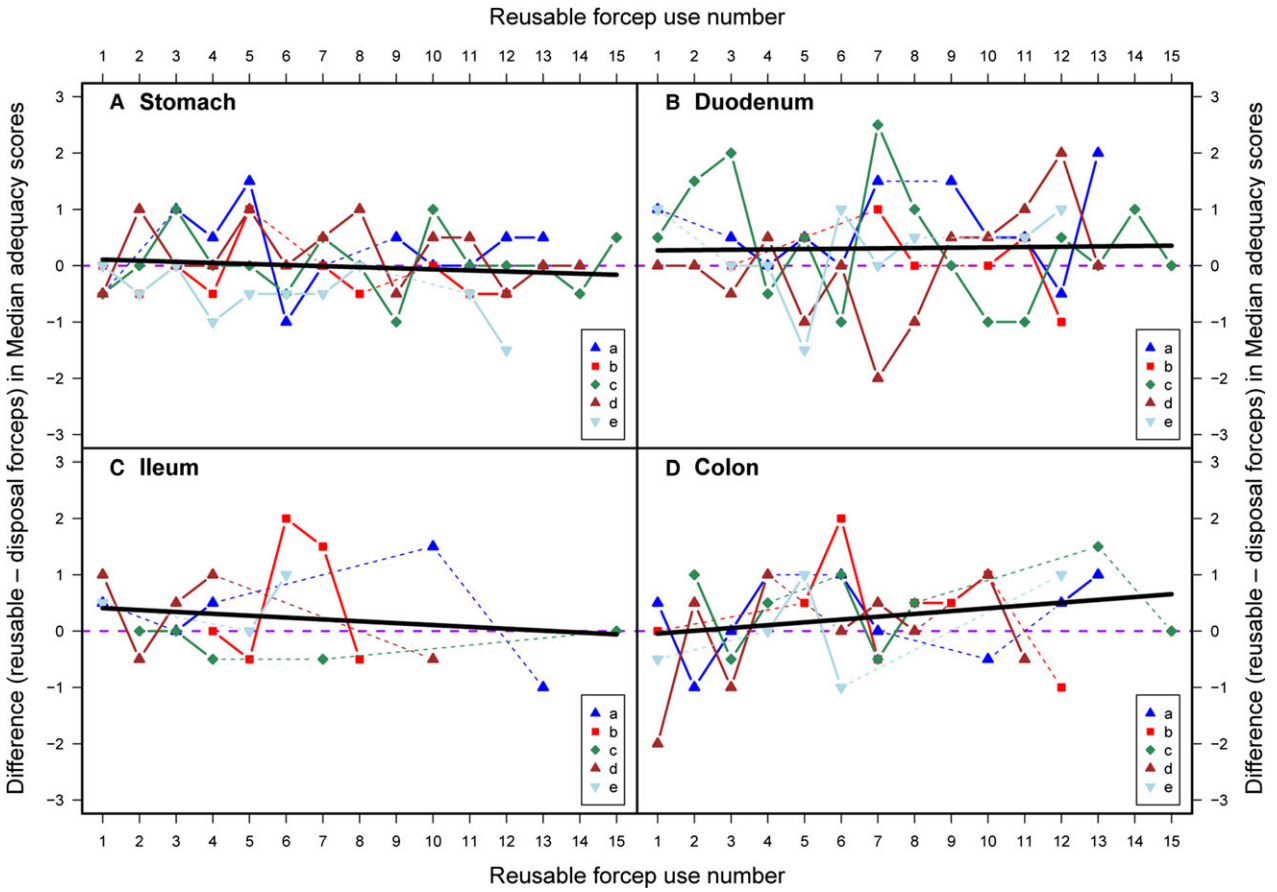

Fig 2. Difference (single-use (disposable)—reusable forceps) in the median adequacy scores obtained from biopsy samples taken from 4 organs—the (**A**) stomach, (**B**) duodenum, (**C**) ileum, and (**D**) colon—versus the number of times 5 reusable forceps a (dark blue), c (red), c (green), d (maroon), and e (light blue) were used. Solid lines indicate consecutive samples obtained, dashed lines join data where an intermediate sample's adequacy could not be determined. Purple dashed line indicates 0 difference, and the black solid line is the fitted regression line from a linear mixed-effect model of difference in the median adequacy scores with use. A positive difference indicates that the single-use (disposable) forceps median was higher.

noted with 1 single-use forceps. Once it was confirmed that no deterioration in biopsy quality had occurred, the reusable forceps were continued to be used in the hospital without further statistical comparison of biopsy quality. At >15 dogs for each forceps (A–E), reports for no sample obtained and difficulty opening were present more frequently and these forceps could not be used for >20 dogs because of mechanical failure.

Discussion

This longitudinal study comparing biopsy sample quality obtained with reusable and single-use biopsy forceps showed no decline in tissue quality when using reusable forceps to collect biopsy specimens from 10 to 15 clinical cases compared to single-use forceps. Alligator-toothed forceps were chosen as they provide adequate quality tissue biopsies[15] and are commonly used in veterinary medicine. Sample quality was utilized for comparison as this is important for diagnostic purposes.[5] Some descriptive evaluations were provided by technician and endoscopist, such as those pertaining to mechanical failure, which did increase with time. This is consistent with the malfunction reported in some human comparison studies. Human studies have compared forceps subjectively, with operators scoring ease of passage through the endoscope and ease with which forceps open[7–9] or size of sample.[8] These subjective measures were not pursued in this study, as this would have introduced significant bias, as it was not possible to blind the operator to the type of forceps.

A previous study in veterinary medicine has compared various single-use forceps types in healthy animals,[12] findings in healthy dogs may not be directly translatable to that of dogs with gastrointestinal pathology. Pathological changes can affect sample quality in people[16] and would be expected to affect sample quality in dogs and cats, although this was not found in this study. Because only two dogs were diagnosed with marked inflammation, the study may have been underpowered to detect the effect of inflammation on adequacy scores.

One endoscopist and technician had improved quality scores compared to the other endoscopists and technicians in 1 section of gastrointestinal tract. This study included a large number of endoscopists and technicians. Although the same biopsy technique and sample handling was used by all endoscopists and endoscopy technicians, experience of both may have affected results. The higher depth and adequacy obtained for the single-use biopsy forceps for 2 endoscopists may be attributable to the inability to blind the endoscopists to forceps type. Some endoscopists expressed a preference for single-use forceps and, as such, might have resulted in an improved sampling technique.

An additional variable that may dictate choice of forceps type is potential for pathogen transmission. Various pathogens have been transmitted by reusable forceps in people; Salmonella species, *Pseudomonas aeruginosa*, Helicobacter pylori, *Strongyloides stercoralis*, hepatitis B virus, and hepatitis C virus.[17] Organic material has also been identified inside forceps despite reprocessing and although transmission of disease is not currently recognized in dogs, there is no current screening for this complication. The use of single-use forceps would likely exclude this risk. Pathogen transmission was not examined in this study. The cleaning protocol used in this study was standardized and did not include autoclave, although this has been shown to be superior.[18] The effect of reprocessing on biopsy sample quality remains unknown, although this is not expected to affect the result of forceps type comparison and we detected no decline in biopsy quality.

Cost of forceps at the authors' institution was £21 per single-use forceps and £200 per reusable forceps. The cost of reprocessing including nursing time and consumables was £0.79 per forceps. Using a new single-use forceps for each dog, the reusable forceps become economically viable at 10 dogs. No decline in biopsy quality was detected compared to single-use biopsy forceps with at least this number of dogs. Although additional use may have demonstrated a decline in quality or more frequent mechanical failure, use of the reusable forceps in over 10 dogs makes this the most cost-effective option while maintaining equivalent sample quality.

Footnotes

[a] Olympus Alligator standard cup, (2.0 mm 1550 mm); Olympus Medical, Essex, SS2 5QH, United Kingdom.

[b] Olympus Lucera XP260 gastroscope, 2 mm biopsy channel; Olympus Medical, Essex, SS2 5QH, United Kingdom.

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Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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Supporting Information

Additional Supporting Information may be found online in the supporting information:

Fig S1. As for Fig 2, but showing difference (single-use (disposable)—reusable forceps) of the median depth scores for the 4 organs.

Fig S2. As for Fig 2, but showing difference (single-use (disposable)—reusable forceps) of the median crush scores for the 4 organs.

Fig S3. As for Fig 2, but showing difference (single-use (disposable)—reusable forceps) of the median number of villi obtained from biopsy samples taken from the duodenum (a) and ileum (b).