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CLINICAL RESEARCH

Comparison of the diagnostic yield of 3 and 5 mm laparoscopic liver biopsy forceps in cats

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Abstract

Objective: To determine whether 3 and 5 mm laparoscopic cup biopsy forceps provide samples of equivalent diagnostic quality in cats.

Study design: Experimental study.

Animals: Twelve colony cats undergoing a concurrent nutrition study.

Methods: Two biopsy forceps (3 and 5 mm) and three biopsy techniques (twist, pull, and twist + pull) were used to collect 68 laparoscopic liver samples. Biopsies were performed consecutively with the 3 and 5 mm biopsy sites adjacent to each other. Data analyzed included the number of portal triads and hepatic lobules, tissue crush and fragmentation, overall sample area (mm^2), sample weight, and agreement regarding morphologic diagnosis.

Results: The 5 mm forceps provided more hepatic lobules, portal triads, and a larger tissue weight and histologic area (mm^2) ($p < .01$). The twist and pull techniques provide more hepatic lobules and portal triads compared to the twist + pull technique while the twist + pull technique resulted in greater tissue crush compared to the twist technique ($p = .0097$). There was good agreement for morphological diagnosis between the 3 and 5 mm samples using the twist + pull technique but not for the twist or pull techniques.

Conclusion: Liver samples can be safely collected with 3 or 5 mm laparoscopic biopsy forceps and provide sufficient tissue for histopathology analysis in cats, with minimal artifact. The diagnostic accuracy of 3 mm samples remains unknown.

Clinical significance: Although 3 mm laparoscopic cup biopsy forceps provided samples of sufficient diagnostic quality for histopathologic interpretation in cats, further studies are required to assess their diagnostic accuracy.

Abbreviations: P, pull; T, twist; TP, twist + pull.

1 | INTRODUCTION

Hepatic lipidosis and inflammatory liver diseases are reported as the most common (49.7% of all such diseases), and second most common (25.7%) hepatic diseases identified histopathologically in feline livers.[1] Liver biopsy is the single most informative diagnostic test for definitive diagnosis of feline liver disease.[2] Larger liver biopsies obtained during surgery or necropsy, such as the guillotine or wedge liver biopsy, are generally considered the gold standard techniques for histologic diagnosis.[3–5] One of the main advantages of these techniques is that the larger sample size allows enhanced diagnostic testing.[6] However, these techniques require a laparotomy or a minilaparotomy, which has disadvantages, namely a larger abdominal incision and longer postoperative recovery times.[5] Vasanjee et al.[7] compared five intraoperative liver biopsy methods in dogs, including the 5 mm laparoscopic cup biopsy forceps and found that all methods produced minimal complications and, aside from the needle biopsy sample, all yielded adequate tissue samples for histology. Current research supports laparoscopy as a safe, minimally invasive procedure that allows abdominal organ visualization and biopsy collection.[8,9]

Five millimeter cup biopsy forceps are regarded as the standard method for laparoscopic tissue sampling in dogs but there are no data specific to cats.[8] Subjectively, the length (36 cm) and cup size of the 5 mm biopsy forceps is large when working in smaller patients with less working space, and results in relatively large tissue samples relative to the organ from which it is retrieved. Newer pediatric laparoscopy equipment, such as the miniature 3 mm biopsy forceps (20 cm long), has the potential to obtain samples of a similar diagnostic quality while minimizing the amount of tissue that is biopsied and can be performed through smaller laparoscopic incisions/portals. In a study utilizing canine cadavers, Kimbrell et al.[10] reported that there was no significant difference in morphologic diagnosis between the 3 and the 5 mm laparoscopic biopsy forceps. As far as the authors are aware, liver biopsy techniques have not been investigated for feline patients.

Several laparoscopic liver biopsy collection techniques have been reported.[8,9,11,12] Anecdotally, a twisting motion while obtaining a laparoscopic liver biopsy is thought to assist with hemostasis and was the primary technique used in several studies.[8,13] Most recently, Buote et al.[11] determined that a 360° twist technique resulted in larger liver biopsy samples and fewer histologic artifacts in canine cadavers when compared to a pull technique and pull through a cannula technique. However, there is limited research in both cats and dogs that directly assesses differing laparoscopic liver biopsy techniques and the quality of samples obtained.

As such, the primary objective of this study was to determine if 3 mm miniature laparoscopic liver biopsy forceps would provide samples of equivalent diagnostic quality in cats compared to standard 5 mm laparoscopic biopsy forceps. A secondary objective was to compare the effect of a twist (T), pull (P), and twist + pull (TP) biopsy collection technique on sample size and quality. The authors hypothesized that liver biopsies collected using the 3 mm miniature laparoscopy biopsy forceps would provide samples of equivalent diagnostic quality for histopathologic analysis compared to standard 5 mm biopsy forceps and that the 3 mm biopsy forceps, and the T technique, would result in equivalent or more tissue fragmentation and crush artifact.

2 | MATERIALS AND METHODS

2.1 | Animals

This study was conducted in accordance with the guidelines of the Canadian Council on Animal Care and was approved by the institutional animal care committee at the University of Guelph (approval number: AUP ##4118). Twelve purpose bred male cats underwent two separate laparoscopic liver biopsy procedures as part of a nutrition study assessing the effects of choline and L-carnitine supplementation on lipid metabolism once in April 2021 and again in August 2021. Biopsies for this study were collected during the laparoscopic procedures for the nutrition study. All cats were clinically healthy at the time of biopsy collection; however, one cat developed urinary signs following the first biopsy event and was removed from the study prior to the second biopsy event.

2.2 | Preoperative bloodwork

The cats were deemed to be clinically normal through multiple physical examinations, serum biochemistry analysis and complete blood counts.

2.3 | Anesthetic protocol

Cats were premedicated with hydromorphone 0.05 mg/kg IM (hydromorphone hydrochloride; Sterimax) and acepromazine 0.04 mg/kg IM (Acevet; Vetoquinol) then induced with alfaxalone 1–3 mg/kg IV (alfaxan; Jurox Pty Ltd) to effect and midazolam 0.3 mg/kg IV (midazolam; Sandoz). General anesthesia was maintained with isoflurane (Fresenius Kabi Canada) in oxygen. Cats received intraoperative meloxicam 0.1 mg/kg subcutaneously

(Metacam; Boehringer Ingelheim) as well as one intraoperative dose of cefoxitin 22 mg/kg IV (Teva Pharmaceuticals).

2.4 | Surgical technique

Cats were placed in dorsal recumbency and the ventral abdomen was clipped, aseptically prepared and draped for abdominal surgery. The surgical procedures were performed by a board-certified surgeon, BB (12 cats, first biopsy event), or one of two surgical residents – (RD) (six cats, second biopsy event) and PCL (five cats second biopsy event).

A modified-Hasson technique was used to introduce a 5 mm threaded trocar canula (Storz) ~1 cm caudal to the umbilicus.[14] The abdomen was insufflated to 6–10 mmHg with carbon dioxide using a pressure regulating mechanical insufflator (Storz) and a 5 mm 30° telescope (Storz) was introduced. Under visualization, an incision was made using a #15 blade in the left lateral abdominal quadrant a few centimeters cranial to the camera portal. A second 5 mm threaded trocar canula was introduced through the incision (instrument portal) and a cursory abdominal exploration was performed; the liver was assessed for gross abnormalities.

Liver biopsies were collected from the edges of the liver lobes, avoiding previous biopsy sites during the second biopsy event. In the first biopsy event, paired biopsies (3 and 5 mm) were collected using a TP technique, totaling two samples per cat. In the second biopsy event, paired biopsies were obtained using a P technique and a T technique, totaling four samples per cat. The order of biopsies (3 vs. 5 mm), and the technique used first for the second biopsy event were determined randomly by a surgical assistant at the time of biopsy collection. Each cat had a total of six biopsies performed between the two biopsy events.

Prior to closure, insufflation was terminated and the trocar canula opened to deflate the abdomen prior to removal. All incisions were closed using interrupted cruciate patterns in two layers (rectus fascia and the subcutaneous tissue) using a monofilament absorbable suture (3-0 PDS; Ethicon) followed by an interrupted cruciate pattern using monofilament nonabsorbable suture (4-0 prolene; Ethicon) for the skin.

2.5 | Biopsy techniques

Three and 5 mm Storz Blakesley cup forceps purchased for this study were used. The biopsy instrument was introduced through the left paramedian trocar canula, using a 3 mm seal cap to prevent loss of pneumoperitoneum when using the 3 mm biopsy forceps. The biopsy site was identified, and the lobe was elevated gently using the closed biopsy forceps to isolate the site from other abdominal organs and retracted slowly to allow the edge of the liver lobe to fall into the jaws of the forceps. The forceps were advanced carefully to seat the liver lobe edge fully into the jaws and the forceps were closed and held for 3–5 s to help with hemostasis.

Three biopsy techniques were used. The TP samples were collected by twisting the forceps 90° clockwise while gently pulling away from the liver lobe until the tissue sample released from the liver.[9,15] The T samples were collected by twisting the forceps 180° back and forth until the tissue sample released from the liver. For the P samples, the forceps were pulled away from the liver lobe until the tissue sample released from the liver.[7,16] Three and 5 mm biopsies were performed consecutively and sampling sites were separated by a few millimeters of grossly normal liver tissue. Immediately after biopsy collection, the surgeon measured each sample in two dimensions (mm^2) using a sterile stainless-steel ruler and the samples were then weighed (Digital Portable Milligram Scale 100 × 0.001 g Newacalox) and placed in a labeled 10% neutral buffered formalin jar.

2.6 | Postoperative care

Cats received postoperative buprenorphine 20 mcg/kg IV (Vetergesic) once on recovery. They were returned to their research colony with an Elizabethan collar and meloxicam (Metacam; Boehringer Ingelheim) 0.1 mg/kg orally once daily for 3 days with gabapentin (gabapentin; Noumed) 10 mg/kg orally every 8–12 h for 5 days.

2.7 | Microscopic analysis

Samples were stored in formalin until they were all collected and then they were trimmed and processed routinely into paraffin for sectioning and staining with hematoxylin and eosin stain by the animal health laboratory at the University of Guelph. Samples were assigned a random identification number and were read in a blinded fashion by a board-certified veterinary pathologist (RF) to determine the number of portal triads and hepatic lobules available per section, to assess for the presence/severity of crush and fragmentation artifact and to determine a morphologic diagnosis. A portal triad was identified by the presence of a terminal bile duct, and a lobule was counted when the terminal hepatic vein and surrounding portal triads were observed. The histologic samples were measured in two dimensions (mm^2) using a surgical ruler as described previously. A scoring rubric adapted from Fernandez et al.[12] was used to assign crush

and fragmentation scores on a scale of 0–3; 0 none, 1 mild, 2 moderate, and 3 severe.

2.8 | Statistical analysis

Friedman test one-way ANOVA was used to assess the differences in scores for fragmentation and crush artifact. A general linear mixed model accounting for the block effect for cats as well as main effects of size and technique, and their interaction, was tested for differences in the sample size (mm^2), sample weight (g), as well as the number of portal triads and hepatic lobules. Residuals were checked for normality using a Shapiro–Wilk test. Hepatic lobules and sample size (mm^2) residuals were not normally distributed and were log transformed to meet the assumptions of normality. When significance was detected in the main effect, a post hoc Tukey adjustment was applied for pairwise comparisons of size by technique interaction. A test of symmetry compared proportions for significant differences. All analyses were performed using a commercial statistical software (SAS Institute Inc. 2013. SAS/STAT® 9.4. Cary, NC) and $p < 0.05$ was considered significant. A weighted kappa test was used to assess agreement for morphological diagnosis, fragmentation and crush artifact for each pair of samples (3 vs 5mm) for each biopsy technique in each cat.

3 | RESULTS

3.1 | Animals and procedures

The median age of the cats was 21 months (range 15–26 months) at the time of the first biopsy event and 24.8 months (range 19–30 months) at the time of the second biopsy event. The median weight was 5.5 kg (range 4.3–7.2 kg) at the time of first biopsy and 5.5 kg (range 4.3–7.1 kg) at the second. Between the two biopsy events there were a total of 68 samples obtained for analysis. Other than the cat that was excluded from the second biopsy event and had a total of two samples collected at the first event only, each cat had a total of six samples obtained. Depending on ease of access, all biopsies were obtained from the left lateral and/or left medial liver lobes. Access to the right liver lobes was limited in larger cats using the shorter 3 mm forceps through a left paramedian portal site.

3.2 | Biopsy sample weight

Thirty-one 3 and 5 mm sample pairs were weighed immediately following collection. Sample weights were not collected for three sample pairs due to a scale malfunction that caused erratic measurements during the first biopsy event. The mean sample weight using 3 mm forceps was 0.056 g (0.02–0.139); using 5 mm forceps it was 0.079 g (0.016–0.158). Forceps size affected sample weight ($p < .01$), with 5 mm biopsy forceps resulting in a heavier sample than the 3 mm forceps (Table 1). No sample weight difference was observed between techniques ($p = .32$).

3.3 | Tissue surface area

Four data points using the 3 mm forceps and three data points using the 5 mm forceps measuring tissue surface area were lost and unavailable from the first biopsy event. Forceps size affected tissue surface area ($p < .0001$) with the 5 mm biopsy forceps resulting in a larger tissue surface area (mm^2) compared to 3 mm forceps (Table 1).

3.4 | Hepatic lobules and triads

There was a mean of 4.9 (4.1–5.9) hepatic lobules for the 3 mm forceps and a mean of 12.4 (10.7–14.3) for the 5 mm forceps (Table 1). The TP technique had a mean of 6.6 (5.4–8.0) hepatic lobules; the T technique had a mean of 9.0 (7.4–10.9), and the P technique had a mean of 8.0 (6.6–9.8; Table 1). Five-millimeter forceps provided more hepatic lobules than 3 mm forceps ($p < .0001$). A technique (TP vs. P vs. T) effect was noted ($p = .016$); the T technique resulted in more hepatic lobules than the TP technique ($p = .005$) but there was no difference when the T technique was compared with the P technique ($p = .25$), or when the TP technique was compared with the P technique ($p = .08$; Table 1).

There was a mean of 19.0 (16.7–21.5) portal triads for the 3 mm forceps and a mean of 29.6 (26.3–33.2) for the 5 mm forceps (Table 1). The TP technique had a mean of 21.0 (18.3–24.0) portal triads, the T technique had a mean of 24.8 (21.6–28.4), and the P technique had a mean of 25.6 (22.4–29.3; Table 1). The 5 mm biopsy forceps resulted in more portal triads than the 3 mm biopsy forceps ($p < .0001$). There was an overall effect of biopsy technique ($p = .007$), with both P and T techniques resulting in more portal triads than the TP technique ($p = .003$ and .015, respectively), but no effect was seen when the T technique was compared with the P technique ($p = .57$). There was a forceps size by biopsy technique interaction on the number of portal triads ($p = .0038$; Table 1), with both the 5 mm P and T techniques resulting in more portal triads than the 3 mm P and T techniques ($p < .0001$). However, the 5 mm

TABLE 1 Mean (95% CI) sample findings for each biopsy technique and forceps size.

	Technique				Forceps size			Size*technique
Biopsy sample characteristics	**Twist + pull**	**Pull**	**Twist**	***p***	**3 mm**	**5 mm**	***p***	***p***
Number of triads	21.0 (18.3–24.0)	25.6 (22.4–29.3)	24.8 (21.6–28.4)	Overall .007 P–TP .003 P–T .57 TP–T .015	19.0 (16.7–21.5)	29.6 (26.3–33.2)	<.0001	Overall .0038 3*5 P < .0001 3*5 TP .14 3*5 T < .0001
Number of lobules	6.6 (5.4–8.0)	8.0 (6.6–9.8)	9.0 (7.4–10.9)	Overall .016 P–TP .08 P–T .25 TP–T .005	4.9 (4.1–5.9)	12.4 (10.7–14.3)	<.0001	Overall .11 3*5 P < .0001 3*5 TP <.0001 3*5 T < .0001
Sample biopsy weight (g)	0.091 (0.021–0.142)	0.137 (0.084–0.158)	0.020 (0.016–0.08)	Overall .32 P–TP .33 P–T .97 TP–T .44	0.056 (0.02–0.139)	0.079 (0.016–0.158)	<.0001	Overall .23 3*5 P < .0001 3*5 TP <.0001 3*5 T < .0001
Tissue area (mm^2)	30.2[a] (25.4–36.0)	34.5 (29.7–40.1)	33.2 (28.6–38.6)	Overall .19 P–TP .08 P–T .65 TP–T .17	20.6[a] (18.1–23.5)	51.6[a] (45.4-58.6)	<.0001	Overall .50 3*5 P < .0001 3*5 TP <.0001 3*5 T < .0001
Histology area (mm^2)	27.7 (23.9–32.0)	33.6 (28.9–39.1)	32.4 (27.9–37.7)	Overall .06 P-TP .03 P-T .68 TP-T .07	20.2 (17.7–23.0)	48.0 (42.0–54.8)	<.0001	Overall .001 3*5 P < .0001 3*5 TP <.0001 3*5 T < .0001

[a]Four missing data sets in sample area 3 mm, three missing data sets in sample area 5 mm.
Abbreviations: P, pull; T, twist; TP, twist + pull.

TP technique did not result in more portal triads than the 3 mm TP technique ($p = .14$).

3.5 | Histologic area

Forceps size affected sample histologic area ($p < .0001$), with the 5 mm biopsy forceps resulting in a larger histologic area (mm^2) than the 3 mm forceps. There was no overall effect of biopsy technique ($p = .06$) on sample histologic area (Table 1).

3.6 | Crush and fragmentation

There was no effect of forceps size ($p = .67$; Table 2) on tissue crush. However, there was an effect of biopsy technique ($p = .03$), with the TP technique resulting in greater tissue crush than the T technique ($p = .01$), but not the P technique ($p = .07$), or when comparing the T technique with the P technique ($p = .41$). Regardless of biopsy technique, all tissue-crush grades were moderate (grade 2) or less and did not hinder the ability to reach a histopathologic diagnosis.

3.7 | Histopathologic diagnosis

Good agreement was found between the 3 and the 5 mm biopsy samples for microscopic diagnosis using the TP technique ($\kappa = .75$). However, there was poor agreement between the 3 and the 5 mm samples for histologic diagnosis for both the P and T techniques ($\kappa = .15$ and $\kappa = .12$, respectively). All samples analyzed, despite size or technique used to obtain them, were considered to be of sufficient diagnostic quality, and received a final histopathologic diagnosis. Diagnoses were distributed equally across biopsy forceps size and were graded as mild, and not considered clinically relevant. Diagnoses included nodular hyperplasia (one case), reactive portal hepatitis (six cases), periportal glycogenosis (one case), periportal lipidosis (six cases) and normal liver (54 cases) (Table 3).

4 | DISCUSSION

This is the first study assessing the use of miniature laparoscopic biopsy forceps for liver biopsy in cats. The results suggest that the liver biopsies performed using the 3 mm miniature laparoscopic biopsy forceps provided smaller samples than the 5 mm biopsy forceps but contained sufficient hepatic lobules and portal triads based on previously reported guidelines to reach a histopathological diagnosis.

Eighty percent of biopsy samples in this study were assessed as histologically normal and all morphologic diagnoses were graded as mild and did not require medical treatment. Periportal lipidosis was identified in 9% of biopsies, and is defined as the accumulation of lipid within the cytoplasm of hepatocytes of the periportal zone.[17] The cats used in this study were undergoing a nutritional study assessing choline and L-carnitine supplementation in lean and obese cats. Choline and L-carnitine affect hepatic metabolism – specifically lipid metabolism – which may have affected fat deposition within the hepatocytes, depending on body fat percentage.[18,19] The diagnosis of periportal lipidosis in this study was therefore unsurprising. Reactive portal hepatitis was diagnosed in 9% of samples. This can result from exposure to various infectious, metabolic, or pharmaceutical agents, and is often diffusely distributed throughout the liver.[17] However, identifying a cause can be challenging through histologic analysis alone.[17]

Although the morphologic diagnosis from 3 mm samples obtained using the TP technique provided good agreement with 5 mm samples, this was not the case for

TABLE 2 Median (range) histologic findings for each biopsy technique and forceps size.

	Technique				Forceps size			Size*technique
Histologic artifact	**Twist + pull**	**Pull**	**Twist**	***p***	**3 mm**	**5 mm**	***p***	***p***
Crush artifact (0–3)	1 (0–2)	0 (0–2)	0 (0–2)	Overall .03 P-TP .07 P-T .41 TP-T .01	0 (0–2)	0 (0–2)	.67	Overall .09 3*5 P .95 3*5 TP .99 3*5 T .80
Fragmentation artifact (0–3)	1 (0–2)	1 (0–2)	1 (0–2)	Overall .62 P-TP .34 P-T .76 TP-T .52	1 (0–2)	1 (0–2)	.22	Overall .57 3*5 P .56 3*5 TP 1.0 3*5 T .99

Abbreviations: P, pull; T, twist; TP, twist + pull.

TABLE 3 Morphologic diagnoses by forceps size and biopsy event.

Biopsy forceps size and event	Nodular hyperplasia	Reactive portal hepatitis	Periportal glycogenosis	Periportal lipidosis	Normal liver
3 mm first event	0	2	0	1	9
5 mm first event	0	1	0	1	10
3 mm second event	1	3	1	1	16
5 mm second event	0	0	1	3	19

the P and T biopsy techniques where poor agreement was noted. This contrasts with results from a study performed by Kimbrell et al.,[10] which reported good diagnostic agreement between the 3 and 5 mm forceps samples in canine cadaver livers, and a study by Buote et al.,[11] which reported excellent agreement between wedge and 5 mm laparoscopic cup biopsies obtained using various biopsy techniques. Care was taken, during paired liver biopsy collection, to obtain side-by-side samples leaving a few mm between samples to avoid iatrogenic artifact that could lead to variations in diagnosis, given that the same area could not be biopsied twice. It is standard practice to obtain three or four samples from differing liver lobes to obtain an overall diagnosis because lesions may not be evenly distributed throughout the entire liver.[16,20] In contrast, in this study, single comparisons between the paired 3 and 5 mm biopsy samples were used, which may also have limited the agreement in this study compared to what might be seen clinically when several samples are collected and pooled to reach a diagnosis. Furthermore, a final definitive diagnosis (typically obtained using a guillotine or at post mortem) was not available in this study. Finally, the TP samples were all obtained from the first biopsy event, which included 12 cats and provided one additional paired sample. It is possible this factor contributed to the agreement observed for this technique and/or the lack of agreement seen for the other techniques where only 11 pairs were available for analysis.

Twenty-five mm^2 is the reported mean surface area of wedge liver biopsies in cats in a single study.[21] Comparatively, the mean surface area of liver biopsies using 3 mm miniature biopsy forceps in the current study was similar at 20.61 mm^2. Thus, obtaining a liver sample using more invasive surgical techniques to provide the largest sample may not be necessary as the 3 mm miniature biopsy forceps may provide a similar sample size. In humans, the number of portal triads is counted to assess if there is sufficient tissue in a sample to make a microscopic diagnosis.[22] Reports range from 6 to 11portal triads necessary for accurate grading and staging of liver disease.[22,23] In dogs, Kimbrell et al.[10] reported that the mean number of portal triads obtained using the standard 5 mm biopsy forceps was 21.4, and 13.8 for 3 mm biopsy forceps. In this study, the mean number of portal triads was larger in the 5mm samples compared to the 3mm samples ($p < .0001$) which was expected considering that the 5 mm biopsy samples will inherently be larger. The mean number of portal triads using 3 mm miniature biopsy forceps was 19, which is notably greater than the recommended 6–11 in humans, and the 13.8 reported by Kimbrell et al.,[10] using the 3 mm miniature cup biopsy forceps in dogs. Therefore, 3 mm miniature cup biopsy forceps theoretically yield enough tissue to provide a microscopic diagnosis, grade, and stage of liver disease. Interestingly, both the T and the P techniques provided more portal triads per sample compared to the TP technique ($p = .015$ and $p = .003$. respectively). It is possible that T and the P techniques result in larger samples with more hepatic architecture. However, the T and P techniques were performed at different biopsy events by different surgeons so it is possible that the results were affected by the biopsy event or the surgeon performing the biopsy.

As far as the authors are aware, there is no study comparing liver biopsy techniques and tissue sample quality in cats. This study found that the TP technique led to greater tissue crush artifact than the T technique alone. This is similar to the results of a recent study that described a twisting technique where 90% of samples had sharp edges and 65% of samples had mild to moderate tearing artifacts.[11] Although the TP technique resulted in greater tissue crush artifact, the median crush artifact was mild (grade 1), and a morphological diagnosis was still obtained in all tissue samples, so this finding may not be clinically relevant.

When the biopsy technique was ignored, there was no overall difference in crush and fragmentation grade between the 3 and the 5 mm biopsy forceps. Both instruments provided a median crush artifact of 0 (none) and fragmentation artifact of 1 (mild), suggesting that both laparoscopic instruments are excellent at limiting liver tissue sample damage and can be used confidently to obtain clinical samples. In contrast, Fernandez et al.[12] reported a median crush and fragmentation artifact of 1 (mild) using the same grading scheme to assess 5 mm biopsy samples in canine patients with hepatic disease.

The grade discrepancy could be related to the sharpness of the laparoscopic instruments used. The instruments used in this study were purchased and used only for this study and a canine clinical liver biopsy study. They were chosen for these studies to ensure the same cup design (no pin) was used for both sampling methods. Discrepancies could also relate to species differences or to the fact that this study included clinically normal colony cats undergoing a nutrition study, whereas the Fernandez et al.[12] study included canine patients undergoing liver biopsies for liver disease which could affect liver friability.

There are several limitations to this study, the most noteworthy being that only one sample was collected with each technique and biopsy forceps at different time points throughout the study. This may have limited our morphologic diagnosis agreement data because clinically several samples from various liver lobes are collected and assessed collectively to reach a diagnosis. This may explain our lower agreement compared to a recent study that examined three samples using each technique and found excellent agreement for the diagnosis.[11] Having three veterinary surgeons with differing surgical experience perform the liver biopsies may also have influenced the size and quality of samples obtained. However, liver biopsies are routinely performed by veterinarians of varying experience, which might lead to some variability with any liver biopsy. Finally, although we performed liver biopsies in live cats for this study, the lack of actual liver disease in the colony cats used for this study may have limited our ability to draw conclusions for a population of cats with clinical liver disease. Future prospective studies looking at the use of the 3 mm miniature biopsy forceps in a clinical population are warranted.

The 3 mm biopsy forceps shaft length was sometimes short in larger cats when used through a left paramedian portal placed slightly cranial to the subumbilical camera portal. This prevented access to all liver lobes, especially those of the right division. Based on this, it may be prudent to place the instrument portal more cranially in larger cats to facilitate access to all liver lobes when using 3 mm laparoscopic cup biopsy forceps.

In conclusion, liver samples can be safely collected with 3 or 5 mm laparoscopic biopsy forceps in cats and provide sufficient tissue, with minimal artifact for histopathology analysis. Based on these results, diagnostic accuracy of 3 mm samples remains unknown. Given a lack of randomization of biopsy technique between surgeon and biopsy event, and only one sample pair collected and compared for each technique, it is challenging to draw solid conclusions regarding the diagnostic accuracy of each biopsy technique. Additional research investigating biopsy techniques and forceps size is warranted to confirm the results presented herein. Moreover, for future research, samples should be collected at the same time points, under the same sampling conditions, with clinical cases, in feline patients.

AUTHOR CONTRIBUTIONS

Dobberstein REA, DVM: Performed surgical biopsies, compiled all data, interpreted data, drafted and revised the manuscript. Brisson BA, DVM, DVSc, Diplomate ACVS: Developed the design of the study, performed surgical biopsies, oversaw data collection, interpreted data, drafted and revised the manuscript. Foster RA, DVM, PhD, Diplomate ACVP: Contributed to the design of the study, performed the histological analysis of all samples and revised the manuscript. Monteith G, MS: Contributed to the design of the study, analyzed data for statistical significance and drafted the manuscript. Larose PC, DVM, DVSc: Performed surgical biopsies and revised the manuscript. Rankovic A, MSc, PhD: Contributed to the design of the study and revised the manuscript. Verbrugghe A, DVM, PhD, Diplomate ECVCN: Contributed to the design of the study and revised the manuscript. Shoveller AK, BScH, PhD: Contributed to the design of the study and revised the manuscript. All authors provided a critical review of the manuscript and endorse the final version. All authors are aware of their respective contributions and have confidence in the integrity of all contributions.

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CONFLICT OF INTEREST STATEMENT

No conflicts of interest have been declared.

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