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Comparison of diagnostic accuracy of laparoscopic 3 mm and 5 mm cup biopsies to wedge biopsies of canine livers

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Background: Diagnostic accuracy of the 3 mm laparoscopic cup biopsy forceps for collection of tissue samples from canine livers is unproven.

Hypotheses/Objectives: Compare sample surface area and portal triad count between 3 mm and 5 mm laparoscopic cup biopsies and compare the histologic diagnosis obtained by each instrument to a standard necropsy wedge. The hypothesis was that more portal triads and greater sample surface area would be found with the 5 mm samples and the laparoscopic instruments would not have significantly different levels of agreement with necropsy wedge diagnosis.

Animals: Twenty-one client-owned dogs undergoing necropsy.

Methods: Prospective ex vivo study. Three samples (3 mm, 5 mm, and wedge) were taken of 2 different hepatic divisions within 24 hours of death. Morphologic diagnosis, World Small Animal Veterinary Association histologic features, surface area, and portal triad numbers were compared among the 3 samples.

Results: There were significantly more portal triads (mean 21.4 versus 13.8; $P < .0001$) and a higher surface area (20.3 mm^2 versus 11.5 mm^2; $P < .0001$) in the 5 mm samples compared to 3 mm samples. Kappa coefficients and percent agreement for histologic diagnosis as compared to the wedge biopsy were not significantly different between the 2 instrument sizes ($\kappa = 0.383$ and 0.436, respectively; 67% and 69%, respectively).

Conclusions and Clinical Importance: Despite yielding smaller sample sizes, the 3 mm laparoscopic cup biopsy has a similar level of histologic diagnostic accuracy to the 5 mm instrument.

KEYWORDS
dog, laparoscopy, portal triad

1 | INTRODUCTION

The diagnosis of most hepatobiliary diseases is most reliably achieved through histopathology of the liver.[1] Cytology can be helpful in diagnosing malignancies or vacuolated diseases but the architecture of the liver lobule cannot be viewed, which limits diagnostic utility.[2] Histologic samples can be obtained in a minimally invasive manner by laparoscopic surgery.[3] Laparoscopic procedures are becoming increasingly common in veterinary medicine especially for diagnostic procedures, with the reported benefits of magnified visualization of the liver, timely identification of hemorrhage, decreased perioperative pain, and accelerated recovery times.[4–7] Diagnostic accuracy and portal triad numbers obtained using less invasive liver biopsy sample methods have been tested. To date, the smallest sampling method that has equivalent histologic agreement (66%) with gold-standard large wedge biopsy is the 14-gauge (14 Ga) needle, when compared to larger sampling

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Abbreviations: WSAVA, World Small Animal Veterinary Association; CI, confidence interval.

methods (5 mm cup and 8 mm punch biopsy instruments).[8] An 18 Ga needle biopsy is a less accurate diagnostic method with a 49% agreement with gold-standard wedge biopsy for the full spectrum of histologic diagnoses.[9] Based on these 2 studies, a minimum of 2.9 portal triads per sample is suggested to yield an adequate diagnostic biopsy, whereas, previously a minimum of 4–6 portal triads per biopsy had been recommended for an accurate diagnosis.[8,9]

With the introduction of pediatric laparoscopy equipment, the 3 mm laparoscopic cup biopsy has been recommended for obtaining liver samples for histopathology in small animals. To the authors' knowledge, no peer-reviewed literature has evaluated the quality and diagnostic capabilities of 3 mm laparoscopic biopsy cup liver samples in dogs. Therefore, whether or not this equipment yields hepatic biopsy samples of similar diagnostic quality to 5 mm laparoscopic biopsy cups instruments remains unknown.

The objectives of this study were to compare sample surface area and portal triad count of microscopic between the 3 mm and the 5 mm laparoscopic cup biopsy and to compare the histologic diagnosis obtained by each laparoscopic instrument to a standard necropsy wedge biopsy. The hypothesis was that a greater number of portal triads and sample surface area would be found with the 5 mm laparoscopic cup biopsy samples versus the 3 mm laparoscopic cup biopsy samples, and that 3 mm and 5 mm laparoscopic cup biopsy samples would not have significantly different levels of agreement with necropsy wedge biopsy diagnosis.

2 | MATERIALS AND METHODS

Client-owned dogs presented to the Oregon Veterinary Diagnostic Laboratory Necropsy Service at Oregon State University from February 2016 through June 2016 were enrolled. Cadavers from dogs that were euthanized or presented for necropsy for reasons unrelated to this study were included. All study samples were obtained within 24 hours of death or euthanasia and had not been frozen. This study was modeled after a previous publication comparing 8 mm punch, 5 mm laparoscopic cup, and 14 Ga needle liver biopsies to gold-standard necropsy wedge biopsies in an effort to facilitate comparison of results between studies.[8]

Biopsy samples were taken through an open celiotomy during routine necropsy. Two liver lobes (each from a different hepatic division) were selected for sampling; if lesions were noted grossly, the biopsy was taken from this region to mimic clinical surgical goals and not to miss any lesions.[10] Each biopsy method (3 mm laparoscopic cup, 5 mm laparoscopic cup, and gold-standard necropsy wedge) was used for each lobe, yielding 2 replicates for each method and a total of 6 liver samples from each dog (Stryker, 5 mm monopolar handle with biopsy forceps [cup and pin], Kalamazoo, Michigan and Stryker, 3 mm monopolar handle with biopsy forceps [cup], Kalamazoo, Michigan). Two liver lobes were sampled because sampling 2 different liver lobes ensures a more accurate histologic diagnosis.[11] The 3 mm and the 5 mm laparoscopic cup biopsy samples were taken from the edge of the liver lobe to approximate clinical laparoscopic sampling technique and performed

by a single individual (R. Olsen) to minimize variation in technique. The necropsy wedge biopsies were obtained as part of the complete necropsy examination, including routine histopathology of the liver, by taking $2 \times 2 \times 1$ cm wedges from the edge of the same 2 liver lobes selected for sampling.[8] The resultant histologic necropsy diagnosis associated with the liver was used as the gold-standard to which study samples (3 mm laparoscopic cup and 5 mm laparoscopic cup) were compared.

Liver biopsy specimens were placed into separate cassettes based on sampling location and then immersed in 10% neutral buffered formalin at room temperature. After 24–72 hours of formalin fixation, samples were processed into paraffin at the Oregon Veterinary Diagnostic Laboratory, and 5 μm thick sections were mounted to glass slides and stained with hematoxylin and eosin after standard operating procedures. The resultant slides were randomly numbered by a technician preparing the slides who retained the key for unblinding at the conclusion of the study (Graphpad Prism 7 for Windows, v7.03, Graphpad Software, Inc., San Diego, California). A single board-certified veterinary pathologist (C.V. Löhr) reviewed each slide without knowledge of which slide came from which dog, the history, or of the necropsy findings until the conclusion of data collection.

The number of complete portal triads for each sample size was recorded. A portal triad in each sample was considered complete when 3 luminal structures (portal vein, hepatic artery, and bile duct) were visible.[12,13] Sixteen histologic features were evaluated for the samples.[1] Briefly, neoplasia was recorded as present or absent. Non-neoplastic features were recorded and scored on a scale of 0–3 with 3 being most severe; these included hepatocellular atrophy, hepatocellular hypertrophy, biliary hyperplasia, ceroid lipofuscin pigment, hemosiderin pigment, canalicular cholestasis, congestion, extramedullary hematopoiesis, vacuolar change, fibrosis, tissue inflammation, lobular collapse, hepatocellular necrosis, thrombosis, and vascular abnormalities. Definitions for all histologic features have been previously described.[1,8,9] A morphologic diagnosis based on the World Small Animal Veterinary Association (WSAVA) Liver Standardization Group's classification of hepatic disorders was established using the gold standard wedge after the history and overall picture of the case was presented to the pathologist. Morphologic diagnoses included categories for normal, vacuolar hepatopathy, neoplasia, primary fibrosis, acute hepatitis, chronic hepatitis, congestion, necrosis, cholangiohepatitis, reactive hepatitis, cholestasis, vascular proliferation/anomaly, and peritonitis. Some samples had more than 1 morphologic diagnosis.

An a priori power analysis was performed using previously published data on 14 Ga hepatic needle biopsies (mean of $2.9 \pm$ standard deviation of 1.3 portal triads), as this represented the smallest biopsy size that yielded a diagnostic number of portal triads without significant diagnostic differences from larger biopsy instruments (8 mm punch or 5 mm cup).[8] The power analysis indicated that a sample size of 12 dogs would yield adequate statistical power (80% with a 2-tailed alpha of 0.05) to detect a difference as low as 0.74 mean portal triads between 3 mm and 5 mm cup biopsy instruments. Based on this power analysis, we chose to collect data from 21 dogs to ensure adequate statistical power.

Linear weighted kappa coefficients were calculated between the 3 mm laparoscopic cup biopsy and necropsy wedge biopsy samples, and between the 5 mm laparoscopic cup biopsy and necropsy wedge biopsy samples, for each WSAVA histologic category. Normality of data was assessed by the D'Agostino & Pearson normality test with results used to select subsequent parametric or non-parametric statistical tests, as appropriate. Normally distributed data are reported as mean ± standard deviation whereas non-normally distributed data are reported as median (range). Surface area of the sample on the slide was compared between 3 mm and 5 mm laparoscopic cup biopsy samples using a Mann-Whitney test. Number of portal triads between the 3 mm and 5 mm laparoscopic cup biopsy forceps samples was compared using a paired t test, after confirming adequacy of pairing using Pearson's correlation coefficient. All statistical testing was performed using commercially available computer programs with a 2-tailed $P \leq .05$ considered statistically significant (Graphpad Software, Inc. and Med-Calc 17.5.5, MedCalc Software bvba, 8400 Oostende, Belgium).

3 | RESULTS

Twenty-one medium and large-breed canine cadavers were sourced for the study. Median age of dogs at time of necropsy was 10 years (range 1–14 years) and median body weight was 22 kg (range 4.1–44 kg). Sex distribution included 1 male intact, 10 male neutered, 3 female intact, and 7 female spayed. The study included a total of 42 liver lobes from 21 dogs (2 liver lobes per dog) with 3 matched samples (3 mm, 5 mm, and wedge) from each division (126 samples total). The 5 mm laparoscopic cup biopsy samples contained significantly more complete portal triads than the 3 mm laparoscopic cup biopsy samples (21.4 ± 7.4 versus 13.8 ± 5.1; $P < .0001$). The 3 mm laparoscopic cup biopsy samples yielded significantly smaller surface areas than the 5 mm laparoscopic cup biopsy samples (11.5 [7.3–47.1] mm^2 versus 20.3 [12.4–64.3] mm^2; $P < .0001$).

Agreement between each laparoscopic biopsy instrument and the standard necropsy wedge for WSAVA histologic criteria categories are reported in Table 1 as Kappa coefficients and percent agreement. Percent agreement between the 3 mm and necropsy wedge and between the 5 mm cup biopsy and necropsy wedge was high (>75%) for the identification of hepatocellular hypertrophy, canalicular cholestasis, extramedullary hematopoiesis, lobular collapse, hepatocellular necrosis, and thrombosis. There was no significant difference between the 3 mm and 5 mm laparoscopic cup biopsy instruments in overall percent agreement with the necropsy wedge sample (67% with 95%CI = 59%–79% and 69% with 95%CI = 62%–76%, respectively) or in kappa coefficients (0.383 with 95%CI = 0.064–0.598 and 0.436 with 95%CI = −0.067–0.729, respectively).

Out of the 126 samples examined, there were 140 morphologic diagnoses. Fourteen of the 140 (10%) samples obtained had more than 1 morphologic diagnosis. Distribution of diagnoses was vacuolar hepatopathy 26% (36/140), normal liver 18% (25/140), congestion 23% (32/140), primary fibrosis 13% (18/140), neoplasia 8% (11/140), acute hepatitis 4% (5/140), vascular proliferation/anomaly 3% (4/140), peritonitis

2% (3/140), chronic hepatitis 1% (2/140), cirrhosis 1% (2/140), and cholangiohepatitis 1% (2/140). The 3 mm and the 5 mm laparoscopic samples yielded similar morphologic diagnoses in 90% (38/42) of cases. The frequency of disagreement in morphologic diagnosis between the 3 mm and the 5 mm laparoscopic samples and the wedge was 13/42 (13% with 95%CI = 17%–45%) and 17/42 (40% with 95% CI = 26%–55%), respectively. The frequency of disagreement of the morphologic diagnosis among the 3 mm, 5 mm, and wedge liver biopsy samples obtained from 2 different liver lobes in the same dog was 5/42 (12% with 95%CI = 2%-22%).

Neoplasia was diagnosed in 4 dogs using the wedge and cup biopsy methods (Table 2). Of the samples that were diagnosed with neoplasia, percentage of positive results were 63% for either laparoscopic method and 63% for the wedge. Neoplasia was seen in both liver divisions in 75% of either the wedge or laparoscopic methods.

Primary fibrosis was identified in 4 dogs. In 2 dogs, both lobes had subcapsular fibrosis in both cup biopsies sizes whereas primary fibrosis or cirrhosis was identified in the wedge biopsies. In 1 dog, subcapsular fibrosis was present in 3 mm and 5 mm cup biopsies from both lobes but only 1 of the wedge biopsies. In another dog, subcaspular fibrosis was identified in the 3 mm or 5 mm cup biopsies from different lobes, whereas neoplasia and hepatitis were diagnosed on wedge biopsies from both lobes. Fibrosis in the wedge biopsies from 1 liver was absent in both the 3 mm and 5mm cup biopsies.

4 | DISCUSSION

The results of our study showed no significant difference in diagnostic agreement for histologic features between the 3 mm and 5 mm laparoscopic cup liver biopsy samples compared to standard necropsy wedge biopsy. Surface area and portal triad counts were both significantly greater in the 5 mm laparoscopic cup biopsy samples compared to the 3 mm laparoscopic cup biopsy samples. Therefore, we suggest that the 3 mm laparoscopic cup biopsy instrument provides an acceptable level of diagnostic accuracy, similar to the 5 mm laparoscopic instrument.

The 5 mm laparoscopic cup biopsy contained a mean of 21.4 portal triads per sample in the present study. This was different than a previously reported mean of 3.4 portal triads per 5 mm laparoscopic cup biopsies taken from close to the center of the left lateral liver lobe.[8] The difference could be due to the peripheral location of the liver sample collection in the present study, compared to a central location in the previous study. In humans, many peripheral, subcapsular portal triads are actually diads.[14] While peripheral versus central laparoscopic biopsies have been compared and found similar in volume of hemorrhage, number of lobules, and portal triads, the edges are used more often clinically in laparoscopic biopsy.[2,11] Having a larger sample size increases the diagnostic capability of a liver biopsy and surface area, and weight of the sample taken peripherally or centrally could be assessed in future studies.[15]

The diagnostic agreement between both laparoscopic biopsy sample sizes and necropsy wedge diagnosis for both WSAVA histologic features and the morphologic diagnoses in the present study were

TABLE 1 Histologic criteria score (WSAVA category) percent agreement and kappa coefficients with associated 95% confidence intervals (95% CI) for 3 mm and 5 mm laparoscopic biopsy samples when compared to the wedge liver biopsy in 21 dogs post-mortem

| WSAVA Category | 3 mm cup | | 5 mm cup | |
	% Agreement 95% CI	Kappa coefficient 95% CI	% Agreement 95% CI	Kappa coefficient 95% CI
Hepatocellular atrophy	45% 29%–61%	0.422 0.234–0.610	53% 36%–69%	0.483 0.291–0.676
Hepatocellular hypertrophy	78% 64%–91%	0.766 0.612–0.929	80% 67%–93%	0.784 0.637–0.931
Biliary hyperplasia	75% 61%–89%	0.427 0.149–0.706	70% 55%–85%	0.406 0.135–0.676
Ceroid lipofuscin pigment	40% 24%–56%	0.766 0.612–0.920	47.5% 31%–64%	0.784 0.637–0.931
Hemosiderin pigment	58% 41%–74%	0.394 0.216–0.572	58% 41%–74%	0.425 0.242–0.609
Canalicular cholestasis	88% 77%–98%	0.172 −0.204–0.547	93% 84%–101%	0.556 0.136–0.976
Congestion	55% 39%–71%	0.427 0.149–0.706	53% 36%–69%	0.406 0.135–0.676
Extramedullary hematopoiesis	80% 67%–93%	0.501 0.302–0.699	78% 64%–91%	0.588 0.398–0.779
Vacuolar change	53% 36%–69%	0.427 0.149–0.706	58% 41%–74%	0.406 0.135–0.676
Fibrosis	50% 34%–66%	0.375 0.185–0.565	63% 47%–78%	0.501 0.298–0.703
Tissue inflammation	75% 61%–89%	0.58 0.345–0.814	68% 52%–83%	0.412 0.139–0.684
Lobular collapse	83% 70%–95%	0.394 0.216–0.572	83% 70%–95%	0.425 0.242–0.609
Hepatocellular necrosis	75% 61%–89%	0.331 0.064–0.598	78% 64%–91%	0.331 −0.0669–0.729
Thrombosis	95% 88%–102%	−0.026 −0.061–0.010	93% 84%–101%	−0.034 −0.083–0.014
Vascular abnormalities	58% 41%–74%	0.172 −0.204–0.547	63% 47%–78%	0.556 0.136–0.976
Mean Agreement	67% 59%-76%	0.383 0.137–0.569	69% 62%-76%	0.436 0.168–0.648

No significant differences in these data were observed between the 3 mm and the 5mm when compared to the wedge.

TABLE 2 Number of samples that were diagnosed with neoplasia in all liver samples obtained with a 3 mm laparoscopic cup, a 5 mm laparoscopic cup, or a wedge

Neoplasia	Cholangiocellular carcinoma	Mast cell metastatic	Neuroendocrine metastatic	Hematopoetic (Lymphoma)
3 mm (n = 42)		2	1[a]	2
5 mm (n = 42)		2	1[a]	2
Wedge (n = 42)	2	2	1[a]	

[a]Denotes that neoplasia was only diagnosed in the liver samples from 1 liver division and not in the other liver division.

comparable to a previous study showing 60% agreement.[8] The WSAVA histologic feature scores compared to the wedge for the 3 mm and for the 5 mm were 67% and 69%, respectively. The morphologic diagnoses agreed with the wedge in 69% for the 3 mm sample and 60% for the 5 mm sample. The 3 mm agreed with the 5 mm in 90% of the samples. A morphologic diagnosis according to WSAVA was added in addition to the histologic features because histologic features are evaluated in isolation. The morphologic diagnosis takes into account the entire histologic assessment and allows for the pathologist to give insight into the overall disease processes within the liver. The differences in agreement with the wedge biopsy were not significant between the 2 laparoscopic instrument sizes either in raw percent agreement or in kappa coefficients, suggesting similar overall diagnostic utility of both instruments.

The detection of neoplasia was identical between the 3 mm and 5 mm cup and agreement with the wedge biopsy was similar in 3 of 4 cases. Metastatic mast cell tumor was found in 1 dog in all sample types from all sample lobes. Lymphoma was found on both sized laparoscopic biopsies in both liver divisions but not in either of the wedges. In another dog, the necropsy wedge showed cholangiocarcinoma and the laparoscopic cup of either size was negative for neoplasia. A final case showed metastatic neoplasia with 3 mm, 5 mm, and wedge biopsy samples in 1 liver lobe sampled, but none in the other liver lobe. Previous studies show that neoplasia, when present, is diagnosed in the majority of liver lobes as most cases of neoplasia in the liver are metastatic or multicentric (ie, lymphoma or histiocytic sarcoma).[11] Taken together, these findings indicate that no single biopsy sample accounts for the lesions of the entire liver and support recommendations for obtaining multiple samples from several lobes.

The diagnosis of fibrosis showed poor agreement in the study with only 2 of 4 dogs showing agreement among all 3 biopsy methods. This is consistent with previous studies showing a 50% sensitivity between 5 mm laparoscopic cup and necropsy wedge for diagnosing fibrosis.[8] Previous human and veterinary studies have described unequal distribution of fibrosis within and between lobes, which might account for the variation.[11,16] Subcapsular fibrosis is more common and more pronounced at the margins of liver lobes and is generally considered an incidental finding.[1] Nonetheless, most of the cases with subcapsular fibrosis showed a clinically relevant fibrotic condition (primary fibrosis or cirrhosis) in the wedge biopsies. In our hands, cup biopsies containing at least 15 triads are large enough that findings of fibrosis should raise suspicion of more extensive and potentially clinically relevant processes. Of concern is that 1 case showed primary fibrosis on the wedge biopsy only. However, fibrosis was not identified in either the 3 mm or 5 mm cup biopsies.

The limitations of this study include the use of 1 pathologist for diagnosis, the laparoscopic instrument design, the use of cadavers, under-representation of specific conditions such as neoplasia within the study samples, and use of only 1 staining technique (hematoxylin and eosin). Simultaneously, having a single pathologist ensured uniformity of histologic evaluation and diagnostic criteria application, which have been shown to vary among pathologists.[8,17] The 5 mm laparoscopic cup design included a pin that the 3 mm laparoscopic cup did not have.

Both laparoscopic instruments were the only type available in their size from the same manufacturer and are used in clinical settings. While the surface area of each instrument was different, perhaps the area of diagnostically relevant architecture was not and the presence of the pin might have led to disruption of the hepatic lobule and decreased the area for continuous tissue architecture analysis. To further characterize the physical differences between samples obtained by each instrument size, a future study could compare the weight of the samples before formalin fixation, which is important to consider when submitting liver samples for metal quantification. Post-mortem changes were attempted to be limited by using only fresh cadavers < 24 hours old and excluding any cases judged to have excessive autolysis by the participating board-certified veterinary pathologist. However, early changes associated with autolysis might have obscured more subtle pathologic processes and the use of live animals might have yielded a higher number of portal tracts.[10] Hematoxylin and eosin were the only stains used and special stains, especially stains for collagen (Trichrome Masson) and reticulin stains could have increased the accuracy of diagnosis. A larger sample size would have included a broader array of histologic diagnoses that could have uncovered a difference in diagnostic utility between the 2 instrument sizes, although portal triad numbers obtained by each instrument suggest adequate sample quality can be had with either sized instrument. Finally, although efforts were made to minimize variation in biopsy technique by having all laparoscopic biopsies performed by a single individual who consciously tried to apply a similar amount of force for each sample, variations in technique might have influenced size and quality of the samples obtained.

In conclusion, this cadaveric study demonstrated similar diagnostic performance of the 3 mm and 5 mm laparoscopic cup biopsy instruments for liver biopsies obtained from client-owned dogs. Importantly, although the surface area and portal triad counts were significantly lower in the 3 mm instrument samples, neither histologic features nor final morphologic diagnosis after WSAVA guidelines was different between the 2 laparoscopic instruments as compared to the standard necropsy wedge. The 3 mm laparoscopic cup biopsy instrument can be used to obtain accurate diagnoses in the liver of dogs despite the significantly lower number of portal triads and lower surface area compared to the 5 mm sample. This smaller size instrumentation could be especially helpful in obtaining liver biopsy samples in small breed dogs.

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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.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

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