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Histopathologic Variation between Liver Lobes in Dogs

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Background: Biopsy of the liver evaluates a small portion of tissue, with inferences made to the entire organ. The method and number of biopsies obtained are tempered by consideration of the risks and benefits. Recommendations often include biopsy of more than one liver lobe, although the consistency of histopathology among lobes in dogs is unknown.

Hypothesis/Objectives: To describe the distribution of histopathologic abnormalities between liver lobes. We hypothesized that discordant results would be evenly distributed among all liver lobes.

Animals: Seventy dogs undergoing necropsy.

Methods: Prospective study. Liver samples were obtained from all lobes. A primary diagnosis was assigned to each liver sample based on the predominant histopathologic abnormality.

Results: In this population of dogs, biopsy of at least 2 liver lobes identified the predominant histologic abnormality in 98.6% of the cases. Ten (14%) of the dogs had ≤3 lobes in agreement and could not be assigned a predominant diagnosis. The same diagnosis was present in 6/6 lobes in 39 (56.5%) dogs, 5/6 lobes in 10 (14.5%) dogs, 4/6 lobes in 10 (14.5%) dogs, 3/6 lobes in 7 (10.1%) dogs, and 2/6 in 3 (4.3%) dogs. The number of discordant results did not differ between the liver lobes.

Conclusion and Clinical Importance: The likelihood of obtaining a sample that is reflective of the predominant histologic abnormality in the liver is increased when multiple liver lobes are biopsied.

Key words: Fibrosis; Hepatitis; Liver biopsy.

Liver biopsy is integral in the diagnosis and management of canine liver disease.[1,2] To accurately diagnose diffuse liver disease, a biopsy specimen must reliably represent the abnormalities throughout the hepatic parenchyma. In several species important lesions are distributed throughout the liver in consistent relationship with hepatic architecture.[3–7] Whereas a good quality biopsy would be expected to reveal most of these lesions, biopsy collects only a small portion of tissue and error associated with nonhomogenous distribution of disease is possible. In humans, hepatopathies considered diffuse can have unevenly distributed histopathologic changes.[3–7] However, caution must be used when extrapolating results in humans to dogs. One of the primary indications for liver biopsy in humans is to assess the degree of fibrosis in livers of patients with chronic hepatitis and cirrhosis, whereas biopsy of the canine liver is typically performed to establish a specific diagnosis. In addition, the etiology and prevalence of liver disease differs between species.

Although it has been suggested that, in dogs, liver biopsies should be collected from more than one lobe,[1] little information is available regarding the distribution of histopathologic changes in the canine liver. Studies of liver biopsies from dogs with portosystemic shunts have demonstrated only small differences between liver lobes.[8,9] However, a study of dogs with other hepatopathies found that 14% of laparoscopic liver biopsies obtained from different sites had discrepant diagnoses.[10] In that study, neoplasia, fibrosis, and other diseases were found in some specimens but not others from the same dog. These findings led the authors of the study to recommend that multiple biopsies be obtained, but the study did not use a systematic approach to biopsy nor did it clearly describe differences between lobes.

The aim of this study was to evaluate the distribution of histologic changes throughout the canine liver by comparing samples obtained from each liver lobe at necropsy. The hypothesis tested was that discordant results would be evenly distributed among liver lobes.

Materials and Methods

The study was approved by the Institutional Animal Care and Use Committee of Virginia Tech. Dogs utilized in the study were patients of the Virginia-Maryland Regional College of Veterinary Medicine (VMRCVM) Veterinary Teaching Hospital (VTH) that died or were euthanized and underwent necropsy between May 2011 and August 2012. Cases were enrolled sequentially as they were presented to the necropsy service. In this prospective observational study, a ventral midline incision was made and a single deep tissue sample of approximately 2 cm × 2 cm × 1 cm was collected by sharp dissection near the center of each of the 6 liver lobes (left and right lateral, left and right medial, caudate, and quadrate lobes) within 3 hours of death. Samples were immediately placed in neutral-buffered 10% formalin, and after routine processing, were cut in 5-μm sections and stained with hematoxylin and eosin. Slides were assigned a random number and presented in a random order to a single veterinary pathologist (KZ) who was unaware of the identity of each specimen. A single standardized microscopic morphological diagnosis and severity (minimal, moderate, or severe) was assigned to each lobe based on the predominant pathologic process following the World Small Animal Veterinary Association (WSAVA) Liver Standardization Group guidelines: no abnormality, neoplasia, cholangiohepatitis, reactive hepatitis, acute

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hepatitis, chronic hepatitis, necrosis, vacuolar degeneration, congestion, hypoperfusion, thrombosis, lymphatic obstruction, cholestasis, nodular regeneration, nodular hyperplasia, cirrhosis, fibrosis, and hepatocellular atrophy.[11] Severity scores were subjective on a scale of 0–3 with 0 representing no change, 1 minimal change, 2 moderate change, and 3 severe change. A microscopic diagnosis of vacuolar degeneration included changes because of lipid and glycogen accumulation as well as because of hydropic degeneration. Vacuolar degeneration was identified by hepatocytes with focal or diffuse swelling and pale or poorly staining cytoplasm.[11] Nodular regeneration was distinguished from nodular hyperplasia by the latter being nonencapsulated and comprised by well differentiated and arranged hepatocytes often with some degree of vacuolar change versus nodular regeneration having a more pronounced capsule and disarrayed plates of hepatocytes often seen in association with fibrosis, variable degrees of accompanying inflammation, and atrophy of adjoining hepatocytes. A diagnosis of hypoperfusion included findings of diminished hepatic portal vein profiles often accompanied by arteriolar reduplication. Vascular anomaly was distinguished by thrombi, ectatic veins, loss of arteriolar profiles, or abnormalities associated with other vascular structures other than typical of portal vein hypoperfusion. Only changes that were classified as moderate or severe were considered in arriving at a primary diagnosis.

If a liver had grossly visible focal abnormalities the dog was excluded from analysis. If more than one disease process of moderate or severe intensity was present within one lobe, the dog was excluded from analysis. Each liver with >3 lobes that had a concordant diagnosis were assigned a predominant diagnosis for that liver. A discordant lobe was defined as a histopathologic finding in a single liver lobe not in agreement with the predominant histopathologic diagnosis. The level of severity of a lesion between lobes with the same histopathologic diagnosis was not considered when determining if a lobe was concordant or discordant. Dogs without a common diagnosis in >3 lobes could not be assigned a predominant diagnosis for the liver and thus were excluded from analysis of discordant lobes.

Statistics

A logistic generalized estimating equations (GEE) analysis was used to compare differences in the number of discordant results among liver lobes from each dog. Differences in proportions of diseases that were present in all lobes were compared using Fisher's exact test. All analyses were performed using commercial software.[a] Significance was set at $P < .05$.

Results

A total of 420 liver lobe samples from 70 dogs were evaluated. One case was excluded because more than one disease was present within a sample. Ten (14%) of the dogs had ≤3 lobes in agreement and therefore did not have a predominant diagnosis. The mean ± SD age of dogs was 9.5 ± 4.1 years, and included 6 males, 26 neutered males, 6 females, and 31 spayed females. Breeds included 18 mixed breed, 6 German shepherd, 5 golden retriever, 5 Labrador retriever, 3 miniature dachshund, 3 Boston terrier, and 2 each of Boxer, Irish wolfhound, Jack Russell terrier, miniature pinscher, Pekingese, and standard poodle, as well as 1 each of 17 other breeds. The primary diagnosis as the cause of death or reason for euthanasia as determined from review of medical records included neoplasia in 16, cardiac disease (congestive heart failure, pericardial effusion) in 15, neurologic disorders in 14, infection in 5, endocrine, hematologic, liver, renal, respiratory, or multisystem disease in 3 each, and unknown in 1 dog. Biochemical profiles were performed in the VTH Clinical Pathology Laboratory within 3 days of sampling in 38 cases. Plasma ALT and ALP activities were greater than the reference interval in 24 (63%) and 32 (84%), respectively, and more than twice the upper limit of the reference interval in 12 (32%) and 22 (61%), respectively, of cases. Both ALT and ALP were greater than the reference interval in 24 (63%) of dogs. Plasma bilirubin concentration was above the reference interval in 15 (39%) of cases, with hemolysis the likely cause in 2 dogs.

Multiple histological diagnoses were found in 30/69 (43.5%) dogs, whereas the same diagnosis was made in all liver lobes in 39/69 (57%) dogs (Table 1). The same diagnosis was present in 6/6 lobes in 39 (57%) of cases, 5/6 lobes in 10 (14%) cases, 4/6 lobes in 10 (14%) cases, 3/6 lobes in 7 (10%) cases, and 2/6 in 3 (4%) cases. The diagnoses that were the most commonly present in all lobes included vacuolar change, diffusely infiltrative neoplasia, and cholestasis, whereas those least frequently identified in all lobes included chronic hepatitis and fibrosis (Table 1). In disease categories with ≥5 cases there was no significant difference in the frequency with which each disease was present within all 6 lobes ($P = .0597$). Of the 23 dogs with an elevation of ALT, ALP activity, or both > twice the upper limit of the reference interval that did not have hemolytic anemia, 13 (57%) had the same diagnosis in 6/6 lobes, 5 (22%) in 5/6 lobes, 2 (9%) in 4/6 lobes, and 3 (13%) in 3/6 lobes. Hyperbilirubinemia, hypoalbuminemia, or both were present in 11 (48%) and 16 (70%) of the 23 dogs with ALT, ALP activity, or both > twice the upper limit of the reference range.

The 10 dogs that did not have a predominant diagnosis were excluded from the following analysis of the 59 dogs where the same diagnosis was present in at least 4 lobes. Based on the prevalence of lesion distribution in these 59 dogs, sampling of any single liver lobe would reflect the most prevalent histopathologic diagnosis within the liver in 54 (92%) of the cases. Sampling of any 2 lobes would result in identification of the predominant diagnosis in 58 (98%) of cases.

The 7 cases of neoplasia included lymphoma (4), histiocytic sarcoma (2), and spindle cell sarcoma (1). Neoplasia was present throughout all lobes in 5 dogs, but in 2 livers the same diagnosis was not present in all lobes. In 1 case of histiocytic sarcoma, neoplastic cells were not found in the quadrate and caudate lobes, and in one liver with lymphoma, neoplasia was identified in all but the caudate lobe (Table 2).

Of the 59 dogs with ≥3 lobes in agreement 20 (34%) dogs had discordant results in 1 or 2 lobes. There were no significant differences between any of the liver lobes in the total number of discordant results ($P = .22$; Table 2).

Table 1. Distribution of histopathologic diagnoses in samples obtained at necropsy in individual liver lobes.

Diagnosis	Cases that had diagnosis in at least one lobe	% in 6 lobes	% in 5 lobes	% in 4 lobes	% in 3 lobes	% in 2 lobes	% in 1 lobe
Unremarkable	24	37.5%	8.3%	4.2%	12.5%	16.6%	20.8%
Vacuolar	23	52.2%	13.0%	8.7%	8.7%	4.3%	13.0%
Fibrosis	11	18.2%			27.3%	18.2%	36.4%
Congestion	10	20.0%		30.0%		30.0%	20.0%
Necrosis	9	33.3%	11.1%			22.2%	33.3%
Chronic hepatitis	8	12.5%	12.5%	25.0%	12.5%		37.5%
Cirrhosis	7	42.8%	14.3%			28.6%	14.3%
Neoplasia	7	71.4%	14.3%	14.3%			
Hypoperfusion	5		20.0%				80.0%
Reactive hepatitis	5			20.0%			80.0%
Cholestasis	2	100%					
Atrophy	1			100%			
Lymphatic obstruction	1			100%			
Nodular hyperplasia	1						100%
Vascular anomaly	1		100%				

Table 2. Distribution of discordant histopathologic diagnoses in individual liver lobes.

Diagnosis (Predominant diagnosis in parenthesis)	Left Lateral Lobe	Left Medial Lobe	Quadrate Lobe	Right Medial Lobe	Right Lateral Lobe	Caudate Lobe
Unremarkable	1 (Lymphatic obstruction)	1 (Vacuolar)	1 (Neoplasia)		1 (Vacuolar)	4 (Hypoperfusion; Vacuolar; Lymphatic obstruction; Neoplasia)
Vacuolar	1 (Unremarkable)		2 (Unremarkable; Cirrhosis)			
Fibrosis	1 (Congestion)	1 (Chronic hepatitis)	2 (Congestion; Unremarkable)		1 (Reactive hepatitis)	1 (Reactive hepatitis)
Congestion				1 (Unremarkable)		
Necrosis					1 (Chronic hepatitis)	1 (Chronic hepatitis)
Chronic hepatitis	1 (Necrosis)					
Cirrhosis	1 (Vacuolar)					
Reactive hepatitis	1 (Chronic hepatitis)					2 (Chronic hepatitis; Neoplasia)
Atrophy				1 (Congestion)	1 (Congestion)	
Vascular anomaly					1 (Congestion)	1 (Congestion)
Total discordant results	6	2	5	2	5	9

Discussion

Results of this study demonstrate variation in the distribution of histopathologic abnormalities between liver lobes. Of the dogs that had a predominant process resulting in a diagnosis in ≥4 lobes, sampling of any single liver lobe would reflect the most prevalent histopathologic diagnosis within the liver in 92%, and sampling of any 2 lobes would result in identification of the predominant diagnosis in 98% of cases. These results support the recommendation to obtain biopsy samples from 2 different liver lobes.[1] Because no differences were seen in discordant results between any of the lobes, those chosen for biopsy may be based on accessibility at the time of biopsy. However, if there is disagreement between the 2 samples, as would have occurred in 19% of the dogs in this study, other clinical information, such as signalment, clinical signs, laboratory abnormalities, and ultrasound findings, would be of particular importance in reaching a diagnosis. In addition, the predominant diagnosis could not be determined in 10 (14.5%) of the dogs reported here, since the diagnoses agreed in ≤3 lobes. These probabilities are applicable only to the study population that was not selected based on clinical or laboratory evidence of liver disease, although samples obtained from dogs with elevated liver enzymes had

similar lesion distribution as those from dogs without significant enzyme elevation. It is possible that different results would be obtained in dogs with a higher prevalence of liver disease and that the recommendation to biopsy at least 2 lobes may not apply.

During ultrasound guided percutaneous needle biopsy the left lateral lobe is most commonly sampled because of its relatively large size and distance from biliary structures.[11] In our population, the left lateral liver lobe had a discordant diagnosis in 6/69 (8.7%) cases, which was not different from other lobes. However, whereas total discordant results did not differ between lobes, the total proportion of discordant results was highest in the caudate lobe. Therefore, biopsy of caudate lobe might be limited to cases where focal lesions are present in this lobe.

Neoplasia was the most common diagnosis present in all lobes, and in all livers with neoplasia, it was present in the majority of liver lobes (Table 1). These findings suggest that the types of diffuse hepatic neoplasia encountered in this study are likely to be correctly diagnosed by biopsy of a single lobe. This conclusion should not be extrapolated to dogs with primary or other types of metastatic neoplasia without further study.

Chronic hepatitis and fibrosis were the diagnoses least commonly present in all liver lobes. These data demonstrate the nonuniform distribution of histologic changes in both of these conditions, and indicate that biopsy of multiple lobes may be necessary for their identification. Conflicting results have been reported in humans about the distribution of fibrosis and inflammation between lobes. Whereas some studies have reported substantial variation in fibrosis[6] and inflammation[12], others have shown minimal or no significant differences between lobes.[13–15] However, the population in this study was not selected for clinical suspicion of liver disease, and the clinical significance of the inflammation and fibrosis in these patients unclear.

In this study, only a single large sample was evaluated from each lobe. This may raise concern for variation in histopathologic results because of intralobe variability. However, in a related study performed in the same population of dogs, the authors found 100% agreement between 2 large samples from 1 liver lobe from each of 70 dogs.[16] This finding demonstrates the accuracy of a single large liver sample in establishing a diagnosis within a lobe.

One limitation of this study is that no predominant diagnosis could be determined in 10 cases (14.5%) that had $\leq$3 lobes in agreement. Biopsy of a single lobe in this population may lead to misdiagnosis if histopathologic changes are extrapolated to the entire liver. However, histopathologic abnormalities identified in the minority of liver lobes may not be of clinical importance. For example, the degree of inflammation or fibrosis that results in clinically relevant liver disease in the dog is unknown. Because this study population included many dogs without clinically important liver disease, it is possible that dogs with clinical signs from liver disease might more consistently have lesions in $\geq$4 liver lobes than what is reported here. Furthermore, the samples in this study were large samples from the center of each liver lobe, and were likely larger than what is practical to collect by biopsy in clinical patients. This method was chosen to avoid artifacts that might be present in samples immediately adjacent to the liver capsule[17] and to ensure that tissue volume did not limit histopathologic interpretation.[18] Because tissue samples from biopsies of clinical patients are typically smaller than those obtained in this study, the proportion of discordant results between liver lobes may be higher in clinical patients. However, it is also possible that biopsy samples from patients with clinically relevant liver disease may have more marked or diffuse histopathological changes that may improve the concordance of diagnoses amongst lobes.

Another potential limitation of this study is reliance on a single pathologist for histopathologic interpretation and assessment of severity. However, use of a single pathologist likely resulted in more consistency in findings when compared with multiple observers.[19] Additionally, morphologic diagnoses were made based on standardized guidelines from the WSAVA Liver Standardization Group which should have further increased the consistency of diagnoses.

An additional limitation of this study was that hematoxylin and eosin was the only stain used for histopathologic evaluation. Our aim with this work was to catalog and compare histologic variations appreciable with this methodology. Whereas use of special stains would have identified with greater assurance the presence of various features described in this work, our choice to only include those changes subjectively considered moderate or severe likely resulted in underreporting of lesions that would have been apparent with special stains. In addition, the lack of special staining limited our quantification and refinement of severity scales, and could have resulted in some inaccuracy. Regardless of what features were or were not appreciated in our evaluation of these tissues, there were detectable variations within the samples described. The lack of special stains precludes certainty in what those features actually represent, but we feel that the consistent evaluation of each sample makes our conclusions valid within these limitations.

A previous study of hepatic lesions present in dogs at necropsy[20] reported a lower proportion of cases with chronic hepatitis (8.5%), vacuolar change (11.5%), and unremarkable (19%) samples as compared to our population (Table 1). This discrepancy is likely due to the use of a single sampling site (left lateral lobe) in the previous study, differences in the critical judgment of different pathologists, and differences in the study populations. The higher proportion of livers with histologic changes in our population may reflect the proportion of dogs that have nonuniform changes throughout the liver and were therefore not accounted for in the previous study. Differences in histopathologic interpretation between pathologists is a well-documented phenomenon and likely contributed to the differences between the prevalence of hepatic changes between studies.[2] In addition, this study was performed at a referral hospital

rather than first opinion practices as in the previous study.

In conclusion, the likelihood of obtaining a sample that is reflective of the predominant histologic abnormality in the liver is increased when multiple liver lobes are biopsied. Additionally, biopsy of a single lobe may result in an unacceptably high proportion of samples that do not demonstrate the predominant histopathologic abnormalities. In this population of dogs biopsy of at least 2 liver lobes identified the predominant histologic abnormality in 98.6% of the cases, therefore, the results of this study support previous recommendation that liver biopsies in the dog be collected from more than one lobe.

Footnote

[a] SAS/STAT® software version 9.2., Cary, NC

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

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

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