A Comparison of Liver Biopsies in Dogs.

Stephanie D. Kemp

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David L. Panciera

Kurt C. Zimmerman

Michael S. Leib

W. Edward Monroe

Otto I. Lanz

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ABSTRACT

Background: The liver biopsy technique in dogs that consistently provides samples adequate for accurate histopathologic interpretation and the variability of histopathology among lobes is unknown.

Hypothesis: Liver biopsy specimens obtained via punch, cup biopsy, and 14 gauge needle biopsy would result in similar histopathologic diagnoses to those found on deeply sectioned samples of liver obtained at necropsy and that discordant results would not differ between lobes.

Animals: Seventy dogs undergoing necropsy.

Methods: Liver specimens were obtained from the left lateral liver lobe with an 8 mm punch, a cup, and a 14 gauge needle. Two larger tissue samples were then collected near the center of the left lateral lobe and used as a histologic standard for comparison. Samples were also obtained from all remaining lobes. Histopathologic features and numbers of portal triads in each sample were compared.

Results: The mean number of

portal triads were 2.9 in needle biopsies, 3.4 in cup biopsies, 12 in punch biopsies, and 30.7 in the necropsy samples. Sixty-six percent of needle biopsies, 60% of laparoscopic cup samples, and 69% of punch samples were in agreement with the necropsy samples, differences that were not significantly different. The corresponding kappa coefficient were 0.59 for needle biopsies, 0.52 for cup biopsies, and 0.62 for punch biopsies. Discordant results did not differ between the liver lobes.

Conclusions and Clinical Relevance: A single biopsy using any of the tested techniques is insufficient for reliable diagnosis of liver disease in the dog. Multiple biopsies from 2 lobes is recommended.

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A. The Liver

a. Anatomy

The liver is positioned directly caudal to the diaphragm with the majority of the organ protected by the ribs and only the most ventral extent protruding caudal to the costal arch.[1,2] It is the largest parenchymal organ in the body accounting for 2-5% of the total body weight in most species.[2,3] The canine liver is composed of 6 distinct lobes. These are the left lateral, left medial, quadrate, right medial, right lateral and caudate lobes.[1] The angle between the right medial and quadrate lobes forms a fossa which contains the gall bladder. The left lateral lobe is the largest and most accessible of the lobes.

The liver is a highly vascular organ with two separate afferent blood supplies.[4,5] The hepatic artery, the first branch to leave the celiac artery, supplies highly oxygenated arterial blood to the liver. Additionally, the portal vein supplies the liver with poorly oxygenated venous blood from the abdominal viscera. The portal vein provides 70 percent of the total hepatic blood flow while the hepatic artery contributes the additional 30 percent.[4,5] The large supply of poorly oxygenated blood to the hepatocytes results in relative hypoxia.

Microscopically the liver is arranged into repeating hexagonal structures called hepatic lobules.[6] At each corner of the lobule is a portal triad composed of a portal vein branch, a hepatic artery branch, and a bile duct.[3,4] A large central vein is present in the middle of each lobule. The hepatocytes adjacent to portal triads are referred to as the periportal

zone. Hepatocytes surrounding each central vein are referred to as the centrilobular zone and hepatocytes between the central vein and portal vessels are called midzonal.[6] Other organizational approaches based upon the microanatomy include portal lobules and liver acinus. In these interpretations the liver is divided into zones based on their proximity to the incoming blood supply. Blood flow progresses from the periphery of the lobule through hepatic sinusoids to the central vein.[5] Zone 1 makes up the area of cells immediately surrounding the axial vessels (hepatic artery and portal vein branches) as they enter the liver. Zone 2 is an intermediate zone that receives blood which has already passed by zone 1 cells. The cells of zone 3 extend from zone 2 to the cells surrounding the central vein. As blood passes toward the central vein much of its oxygen is removed by the zone 1 and zone 2 hepatocytes creating relative hypoxia of the zone 3 hepatocytes.[5] Additionally, hepatocytes of zone 3 receive a blood supply that has been depleted of nutrients and charged with metabolites.[5]

In contrast, bile flow progresses in the opposite direction proceeding from zone 3 towards the portal area. Bile is synthesized by hepatocytes and secreted into a network of small ducts called bile canaliculi.[7] The canaliculi are formed by 2-3 adjacent hepatocytes and are lined with microvillus processes from the apical hepatocyte membranes.[7] The canaliculi anastomose and become progressively larger before terminating as hepatic bile ducts in portal triads.[5,7]

b. Relevant physiology

The liver performs a large array of vital functions including metabolism and removal of blood born toxins, assimilation of nutrients, protein synthesis, carbohydrate and lipid homeostasis, as well as bile salt and cholesterol formation.[5]

Hemostasis

One particularly vital role of the liver is control of hemostasis, aberrations of which may have profound clinical consequences. Hepatocytes are responsible for many pro-coagulant functions such as synthesis of all coagulation factors with the exception of von Willebrand factor and factor VIII.[8] Additionally, activation of vitamin K dependent coagulation factors II, VII, IX, X, and protein C take place in the liver.[8] The liver also has a role in the balance of coagulation as it is also responsible for generation and clearance of major anti-coagulant factors such as antithrombin, antiplasmin, and plasminogen.[8] Furthermore, bile secretion into the small intestine aids in digestion and absorption of lipid soluble vitamins such as vitamin K,[9] which is vital in the production of vitamin K dependent coagulation factors.

Liver dysfunction is commonly reported to result in a complex and insufficiently understood coagulopathy in both humans and veterinary species.[8,10-12] One study reports that 57% of dogs with liver dysfunction have at least one abnormal coagulation parameter.[12] Both humans and dogs with chronic liver disease are at increased risk for hemorrhagic or thrombotic complications as a result of an improper pro-coagulant and anti-coagulant balance.[10,13,14] When liver function is compromised it may result in a hypocoagulative or hypercoagulative state due to decreased production or decreased degradation of both pro and anti-coagulation factors.[10,14] Additionally, severe cholestatic

liver disease or biliary obstruction can result in reduced bile acid secretion and impaired intestinal absorption of lipid soluble vitamins such as vitamin K.[9] Subsequent vitamin K deficiency leads to a bleeding diathesis by decreased activation of vitamin K dependent clotting factors.[9] Furthermore, studies suggest that coagulation abnormalities in humans with chronic liver disease may not be directly due to liver dysfunction but rather may be attributable to complicating factors such as bacterial infection, hemodynamic abnormalities, or renal failure.[14-16] However, these complicating factors have not been investigated in dogs with liver disease.

In addition to abnormalities in secondary hemostasis, a mild to moderate thrombocytopenia is commonly reported in both humans and dogs with chronic liver disease.[8,17] The reason for the reduction in platelet numbers is poorly understood, but it has been speculated that several processes may play a role. These include hypersplenic thrombocytopenia [18], decreased thrombopoietin synthesis[19], and anti-platelet antibody production.[20] It has been shown that dogs with chronic hepatitis and concurrent cirrhosis are more likely to be thrombocytopenic than dogs with other types of liver disease, however the mechanism for this has not been elucidated.[12] These data may suggest that risk of hemorrhage after a liver biopsy may be higher in dogs with both chronic hepatitis and cirrhosis.

Along with reduced platelet numbers, dogs with liver disease have decreased platelet aggregation in response to collagen and arachidonic acid, which may contribute to the hemorrhagic complications of liver disease.[21] Thrombocytopenia was reported to correlate with hemorrhagic complications after ultrasound-guided biopsies of abdominal

or thoracic organs.[22] However, the prevalence of dogs with liver disease in the study was not reported.

Hemostasis is commonly evaluated by measuring prothrombin time (PT) and partial thromboplastin time (PTT). Dogs with hepatic disease have been reported to have abnormalities in PT and PTT in 50% and 40%-75% of patients, respectively.[23,24] Furthermore, measurement individual coagulation factor activity, thrombin clotting time, platelet activity parameters, D-dimers, anti-thrombin, and protein C activity has shown that as many as 57% - 93% of dogs with hepatic disease have at least one abnormal coagulation parameter.[12,23] Studies in humans have reported a lack of correlation between abnormalities in coagulation profiles and clinical bleeding after liver biopsy procedures.[25-27] In contrast, Bigge et. al[22] reported that dogs with a prolonged PT were more likely to have bleeding complications after ultrasonographic guided biopsy of various abdominal structures. However, this study also reported that the likelihood of complication after biopsy of the liver was significantly lower than for biopsy of either kidney. Interestingly the most significant correlation between bleeding complications and ultrasonographic-guided biopsy occurred when thrombocytopenia was present rather than prolonged clotting times. However, in some cases thrombocytopenia was quite severe, making hemorrhagic complications unsurprising.

These data suggest that it is difficult to properly assess the coagulation status of both humans and dogs with liver disease, and that the coagulopathy of liver disease is likely multifactorial. Furthermore these studies show that it is difficult to predict which patients are at high risk for major bleeding complications following liver biopsy procedures.

Therefore, it is advisable to choose the method of liver biopsy that is likely to minimize hemorrhage.

c. Histopathology

The knowledge of normal hepatic microanatomy is extremely important for interpretation of a liver biopsy. Lesions affecting specific portions of the hepatic lobule may provide information regarding the etiology and severity of the liver disease. The anatomic distribution of lesions is categorized as focal, multifocal, zonal, locally extensive, or massive.[6] The liver's unique pattern of circulation can result in lesions being unevenly distributed through different zones of the hepatocytes. Hepatocytes in zone 1 are situated nearest to blood vessels and receive a disproportionately large amount of the oxygen and nutrients delivered to the liver.[4] Additionally, these cells are most severely affected by some toxins as they initially receive the highest concentration of bloodborne toxins. Compounds that are directly toxic to hepatocytes such as allyl alcohol and cocaine result in zone 1 hepatocyte necrosis.[28] In contrast, zone 3 hepatocytes receive a blood supply that has a high concentration of metabolic waste products and has been depleted of oxygen and nutrients[4]. This poor quality blood supply results in an increased sensitivity of zone 3 three cells to systemic hypoxia, malnutrition, or toxic metabolites[4]. Zone 3 hepatocytes are most susceptible to toxins that require activation such as carbon tetrachloride and acetaminophen.[28] These toxins are first activated by reactions in zone 1 and zone 2 hepatocytes before exposure to zone 3 hepatocytes where they exert toxic effects.[28]

Additionally, due to the opposite direction of biliary flow within the liver lesions at the periphery of the lobule (zone 1) are more likely to result in cholestasis and icterus than those in the center (zone 3).[5]

The histologic appearance of liver lesions may also give an indication as to the duration, and reversibility of the liver damage.[6,29] For example, hepatocellular swelling and degenerative changes are reversible changes that result from cellular membrane damage. If the inciting cause is removed, the cells are able to return to normal structure and function. Cellular swelling and degeneration can be caused by a wide variety of toxic, hypoxic, and metabolic etiologies.[6,29] Alternatively, histopathological findings of hepatocellular necrosis or hepatic fibrosis are irreversible changes.

The World Small Animal Veterinary Association (WSAVA) has standardized the histopathologic diagnosis of liver disease and has divided liver disease into four basic groups: vascular liver disorders, biliary tract disorders, parenchymal disorders, and neoplastic disease.[30]

Vascular liver disorders

The vascular disorders of the canine liver include portosystemic shunts, outflow disturbances resulting in hepatic congestion, and portal hypertension.[31] These conditions result in histologic lesions consistent with portal vein hypoperfusion or hepatic congestion. Inadequate portal blood flow into the liver from portal hypertension or intra- or extrahepatic portosystemic shunts results in stereotypical histologic appearance most prominent within the periportal regions of the liver lobule.[31] The hepatic arteries or

arterioles become more torturous (hypertension) with increased prominence and reduplication while portal vein branches become smaller and less distinct as do the central veins. Hepatic parenchymal changes include hepatocellular atrophy, increased proximity of portal triads, lymphatic dilation, and in some cases lipogranuloma formation, with sinusoidal dilatation and mild congestion.[31] In acquired shunts portal chronic hepatitis with portal fibrous and biliary hyperplasia are also commonly seen.

Congestion of the liver is characterized by more severe dilation of hepatic veins and centrilobular sinusoids.[31] Chronic hepatic congestion can eventually result in hepatocyte death and fibrous tissue deposition within the extracellular matrix.[31]

Biliary tract disorders

Canine biliary disorders include biliary cystic disease or atresia, cholestasis, cholangitis, or disease of the gall bladder.[32] The hallmarks of biliary disease include changes in portal tracts such as portal inflammation, portal fibrosis, and biliary hyperplasia. Although these changes may be suggestive of a primary biliary disease they may result from other liver pathologies as well. Cholestasis is identified as by the presence of bile pigment in the hepatic parenchyma. This may manifest as bile plugs in the hepatic canaliculi, phagocytosed bile plugs within hepatic phagocytic cells, and bile granules within the cytoplasm of hepatocytes.[32]

Parenchymal disorders

Canine parenchymal liver diseases encompass a wide variety of etiologies including metabolic, infectious, genetic, and idiopathic disorders. Canine parenchymal liver

diseases have been classified into seven categories: reversible hepatocellular injury, hepatic amyloidosis, hepatocellular death, hepatitis, hepatic abscesses and granulomas, metabolic storage diseases, and miscellaneous conditions.[33] Changes to the hepatocytes themselves are frequently the hallmarks of parenchymal disease. Common hepatocellular changes include cellular swelling, vacuolation (glycogen, water, or lipid), amyloid accumulation, intracellular organisms, and cell necrosis.[33]

Reversible hepatocyte injury is associated with hydropic cell and organelle swelling. Other causes of hepatic cell swelling include steroid induced hepatopathy (glycogen accumulation) and hepatocellular steatosis (lipid accumulation).[33] For any of these, the histologic changes may be focal, diffuse, or zonal and can vary greatly in severity and are referred to collectively as a vacuolar hepatopathy.[33] These hepatopathies represent a non-specific light microscopic change which may be due to a variety of systemic conditions such as endocrine disease, and metabolic derangements as well as hepatic ischemia/hypoxia, hepatotoxins, metabolism of xenobiotics and direct physical hepatic trauma .

Hepatic amyloidosis is an irreversible accumulation of protein within the liver. Three of the most common forms of this protein are amyloid-associated (AA), amyloid light chain (AL), and amyloid β (AB). Protein accumulation may be due to chronic inflammatory conditions or a familial predisposition (Chinese Shar-pei dogs, Abyssinian, Siamese, and Oriental cats). Histologically with hematoxylin and eosin stain, amyloid deposition appears as hyalinized pink-red material which collects in the subendothelial space between the sinusoid and the surface of the hepatocyte (space of Disse). [33,7] Other

changes such as hepatocellular atrophy and dilated sinusoids are frequently present as well.[33]

Hepatocellular death occurs through cellular apoptosis or necrosis. Histologically, apoptotic hepatocytes are shrunken, with eosinophilic cytoplasm, and condensed nuclei surrounded by an empty halo.[34] Apoptosis is a capsase mediated process of "programmed cell death" and may be triggered by any number of primary inciting events such as hypoxia, toxins, infectious agents, or immune derangements.[34] Apoptosis results in cell shrinkage and death without loss of the cell membrane integrity, whereas cell necrosis results in cytoplasmic swelling with a loss of membrane integrity.[34]

Inflammatory liver disease can be classified as reactive, acute, or chronic based the morphologic changes accompanying the inflammatory cell infiltrate. Reactive or non-specific hepatitis is characterized by neutrophilic or mixed inflammation with predominance of mononuclear cells in portal areas and surrounding hepatic parenchyma without necrosis.[34] This is a diffuse, mild, non-specific change, which can occur secondary to a distant inflammatory disease, or may be a residual change after previous hepatic inflammation.[34]

Acute hepatitis is characterized by a combination of inflammatory cells with neutrophils in majority, hepatocellular apoptosis and necrosis with or without regeneration.[34] Acute hepatitis typically results in diffuse histologic changes and may be due to multiple etiologies such as infectious agents, hepatotoxins, or drug reactions. Chronic hepatitis is histologically characterized by a combination of hepatocellular apoptosis or necrosis with variable mononuclear or mixed inflammation, regeneration and fibrosis.[34] Typically no

underlying cause can be identified, however rarely cases have been reported secondary to canine infectious hepatitis virus,[35] leptospirosis,[36] or anticonvulsant medications.[37] Additionally, chronic hepatitis may result from excessive hepatocellular copper accumulation. In Bedlington Terriers primary hepatic copper accumulation is due to an inherited genetic defect in hepatocellular copper excretion.[38] Copper accumulation in affected dogs begins in centrilobular hepatocytes and progressively leads to hepatocellular inflammation and death.

Cirrhosis is the end-stage of hepatic inflammation characterized by fibrosis, nodular regeneration, distortion of normal hepatic architecture with loss central veins, and the presence of porto-central vascular anastomosis.[34] The structural abnormalities compromise liver function and increase resistance to blood flow through the liver resulting in portal hypertension and frequently severe clinical signs.

Hepatic abscesses and granulomas are focal or multifocal collections of inflammatory cells typically secondary to infectious agents.[39] Abscesses contain a neutrophilic exudate and are frequently a result of bacterial organisms when seen in association with a multifocal random distribution, however necrotic neoplasms may also result in abscess formation.[39] Hepatic granulomas are characterized by accumulations of inflammatory cells (predominantly macrophages, with fewer intermixed lymphocytes, plasma cells), possibly with fibroblasts or surrounding collagen fibers.[39] A wide variety of bacteria, fungal, and parasitic organisms have been reported to cause hepatic granulomas.

Hepatic metabolic storage diseases result in a numerous of histologic abnormalities, secondary to congenital or acquired metabolic enzyme deficiencies.[39] The most common

abnormality is the presence of vacuoles within hepatocytes, Kupffer cells, and macrophages.[39] The vacuoles are frequently clear however they may also contain hyaline material, or pigmented granules. In severe cases inflammation, necrosis, or cirrhosis may eventually develop. Although vacuolated hepatocytes may be readily apparent, the presence of vacuoles does not distinguish between causes of vacuolation (i.e. storage disease, lipid, glycogen, water)

Neoplasia

Both benign and malignant neoplasms occur within the liver and histopathology is frequently required for differentiation. The appearance of hepatic neoplastic cells can vary from benign, well differentiated cells to malignant, irregular cells exhibiting bizarre features and an infiltrative growth pattern. Neoplastic disorders of the canine liver have been grouped into seven categories: hepatocellular neoplasia, cholangiocellular neoplasia, hepatic carcinoids, primary vascular and mesenchymal neoplasia, hematopoietic neoplasia, and metastatic neoplasia.[40] Metastatic neoplasia is the most common hepatic cancer.[40]

B. Diagnosis of liver disease

a. History and physical examination

The initial physical examination findings in a patient with liver disease are variable and frequently non-specific.[8] Clinical findings that should raise a clinical suspicion of liver disease include signalment, gastrointestinal signs (vomiting, anorexia, diarrhea), ascites,

icterus, neurologic abnormalities, polyuria and polydipsia, or signs of gastrointestinal hemorrhage.[8]

b. Biochemical testing

Many biochemical tests have been developed for the characterization of liver disease. Initial suspicion of liver disease often occurs when elevated enzyme activity originating from hepatocytes and biliary cells such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and γ-glutamyl transpeptidase (GGT) is found.[8,41] Elevation of liver enzyme activity is a sensitive indicator of liver disease but unfortunately many of the enzymes have a low specificity for identification of hepatocellular damage. For example AST is not only stored within liver cells but also has a high concentration within muscle tissue as well as kidney, brain, and erythrocytes.[42,43] Aspartate aminotransferase activity is known to increase not only with hepatocellular injury but also with muscle damage and strenuous physical activity. Isoforms of ALP present in canine serum include liver, bone, and corticosteroid-induced with the liver fraction being the main contributor in health .[44] Increased ALP activity is a common finding in canine cholestatic liver disease[45] however bone growth[8], osteolytic disease[46], glucocorticiods[47], and anticonvulsant administration[47] can all result in elevated ALP activity independent of liver disease. In one report, the sensitivity of elevated serum ALP for hepatobiliary disease in the dog was 75–96%, however specificity was only 51%.[45] Alanine aminotransferase is a liver-specific hepatocyte cytosolic enzyme that is released into serum with hepatocellular membrane leakage,[8] however in the rare disorder of canine X-linked muscular dystrophy ALT activity can be elevated due to muscle necrosis

as well.[48] In liver disease ALT activity reaches its highest activity in cases of hepatocellular necrosis or inflammation, and elevations in ALT activity are roughly proportional to the severity and locality (diffuse disorders associated with higher levels) of liver damage. However, the degree of elevation is not representative of remaining hepatic function and is not useful for predicting the etiology or prognosis of the disease.[8,49]

Furthermore, liver enzyme activity may be artifactually decreased or elevated when liver function is compromised. Metabolism of hepatic transaminases occurs within hepatocytes, therefore when hepatocyte dysfunction is present transaminases may avoid breakdown and circulate in plasma at elevated levels.[50] In contrast, chronic severe end-stage liver disease may result in enzymes that are within reference ranges or only mildly elevated due to replacement of hepatocytes by fibroblasts.[8]

In addition to being a non-specific indicator of liver disease, hepatic enzyme activity also does not provide meaningful information about liver function.[41,49,51] Liver function in dogs is commonly evaluated biochemically by examining concentrations of serum glucose, albumin, urea, hepatic coagulation factors, cholesterol, bilirubin, bile acids and plasma ammonia. While these bioassays can indicate deficits in liver function they are unable to differentiate between categories of liver diseases[41,52] and therefore cannot guide therapeutic decisions.

The current literature suggests that while biochemical testing may be sensitive for identification of liver dysfunction, histopathology of liver tissue is almost always required to differentiate between categories of liver disease.

c. Imaging of the liver

i. Radiography

Radiographic changes in liver size are known indicators of hepatic disease.[53,54] Radiographic indications of hepatomegaly include rounding and blunting of the caudal liver margin, extension of the liver lobes caudal to the costal arch, and caudal displacement of the gastric axis. In contrast microhepatica will result in cranial displacement of the stomach.[55]

Unfortunately, there are several factors that make radiographic liver size and shape difficult to accurately interpret. To adequately visualize hepatic size and shape abundant omental and falciform fat is necessary for contrast.[53-55] Emaciation or abdominal effusion may prevent radiographic evaluation of the liver from loss of abdominal contrast.[53,55] Thoracic conformation may lead to superimposition of the liver margin and costal arch making interpretation difficult.[56] Additionally, normal liver size and shape is quite variable between dogs.[53] It has been shown that dogs with similar thoracic conformations can have great variability in radiographic liver size.[56]

Although radiography may reveal evidence of liver disease, the variability in findings limits its usefulness in the evaluation of canine liver disease. Furthermore, even though radiographs my show changes indicative of liver disease, these abnormalities are non-specific for the etiology of disease and cannot be used to predict the histologic diagnosis.

ii. Ultrasonography

The majority of the normal canine liver can be evaluated sonographically with sub-costal and intercostal views.[57] Hepatic features typically examined during abdominal ultrasonography include parenchymal echogenicity, parenchymal uniformity, vascular structures, biliary structures, and subjective liver size.[57-60] Sonographically the liver is typically slightly hyperechoic when compared to the renal cortex and hypoechoic when compared to the spleen.[55] Furthermore, ultrasonography is an excellent means of assessing the gallbladder and biliary system.[61]

Sonographic changes in hepatic features of size, shape, echogenicity and uniformity may indicate the presence of hepatic disease. For example, ultrasonography is a highly sensitive means of diagnosing hepatic macrovascular abnormalities.[62,63] Ultrasonography has proven to be both highly sensitive and specific for the identification of portosystemic shunting anomalous vessels in dogs.[62-64] Furthermore, ultrasound is valuable for the identification of focal or multifocal hepatic parenchymal lesions.[65-68] It has been shown in humans that ultrasonography can detect approximately 80 percent of hepatic structural lesions larger than 2 cm.[69] Additionally, in cases of canine hepatic neoplasia, several clinically useful sonographic parenchymal patterns have been identified.[66] One study reported that the sonographic presence of a hepatic mass larger than 3 cm was predictive of neoplasia, while the presence of a nodule smaller than 3 cm was predictive of vacuolar hepatopathy.[70] Another study reported that sonographic findings of a focal liver lesion and the presence of peritoneal effusion are predictive of malignant disease.[71] Additionally, in that study the likelihood of malignant disease went up as the size of the focal lesion increased.[71] In the case of canine biliary disease, ultrasonography has been

shown to be invaluable for the identification of multiple disorders such as biliary obstruction, cholelithiasis, and gallbladder mucoceles.[72-75]

However, ultrasound examination of the canine liver does have limitations. Sonographic changes are rarely pathognomonic for an underlying disease and hepatic histopathology is almost always necessary for diagnosis.[57,66,76] Additionally, the liver may be sonographically unremarkable even in the presence of severe disease. This is particularly true in the case of diffuse liver disease.[59,61,68,76,77] A recent study comparing hepatic sonographic findings to histopathology concluded that there is marked variability in the sonographic appearance of liver lesions and no association between sonographic appearance and histopathology findings.[78] Despite the high degree of accuracy of ultrasonography for diagnosis of some types of diffuse liver disorders in humans,[79-82] the same is not true for diffuse disease in dogs.[59,76,83] Numerous reports in dogs have documented sonographically unremarkable hepatic parenchyma in the presence of severe diffuse disease such as neoplasia and cirrhosis.[59,76,77,82].

The current literature suggests that while ultrasonography is a valuable and sensitive tool for the diagnosis of some liver diseases, it has serious limitations in the identification of diffuse liver disease. In many patients histopathologic examination of the liver is required for diagnosis.

d. Sampling of the liver

Histopathologic examination of liver tissue is imperative for the diagnosis, treatment, and prognosis of canine liver disease. For this reason, safe and accurate acquisition of liver

biopsies is paramount. Histopathologic evaluation of liver biopsy samples provides information about the potential cause, chronicity, and reversibility of disease.[84-87] This information is used to predict prognosis as well as to devise a treatment plan for the patient. Unfortunately liver biopsy is an invasive procedure that carries risk.[22,88,89] When considering a liver biopsy, a clinician must balance the risks against the benefits for the patient. Using the least invasive method of liver biopsy that provides an accurate diagnosis minimizes risks.

Fine needle aspiration

Fine needle aspiration of the canine liver is a sampling procedure in which a small gauge (22G – 25G) needle is passed percutaneously into the liver parenchyma for collection of cells. Needle aspirates are frequently guided with concurrent sonographic imaging, but blind and laparoscopic aspiration techniques have also been described.[90-92] Frequently, ultrasound guidance is used to collect cytologic samples from sonographically identified focal lesions.[90-93]

Percutaneous sampling of liver through fine needle aspiration is a relatively safe and minimally invasive method to obtain hepatic cells for microscopic cytologic evaluation.[90,94,95] A liver aspirate can be performed quickly, with minimal risk to the patient, and typically without sedation or anesthesia. However, fine needle aspirates only collect a small number of cells and the accuracy of liver aspirates as a diagnostic test have been questioned. For example, it has been reported that liver cytology aspirates provided an accurate diagnosis in about 30% of dogs when compared to histopathologic diagnoses.[94,96,97] While some cytologic cellular arrangements are useful in classifying

neoplasms[98,99], frequently cytology aspirate samples do not provide enough cellular architecture to establish an accurate diagnosis.[94,100] Furthermore, the sensitivity of liver cytology aspirates for the detection of hepatic inflammation is variable and dependent on the type of inflammation present.[100,101] In both dogs and cats, fine needle cytology aspirate of the liver may be sensitive for the diagnosis of hepatic lipidosis, however it lacks specificity.[94,95] Multiple reports in humans and dogs suggest that hepatic cytology aspiration is most useful in diagnosing neoplastic disease, although the sensitivity of this method has not been well defined.[93,97,101] Finally, hepatic fibrosis, a clinically important feature for determining the severity and reversibility of liver disease, is insufficiently recognized on hepatic cytology aspirate samples.[101]

Overall, though cytology aspiration of the liver is generally non-invasive, it has serious limitations and liver biopsy is indicated in most cases of persistent or severe liver disease.

Tru-cut needle biopsy

Multiple methods for needle biopsy for histopathologic examination of the canine liver have been described including blind[102,103], keyhole, ultrasound guided[104], laparoscopic assisted[105], and surgical.[84] Percutaneous needle biopsy is the least invasive method of canine liver biopsy. This common clinical technique is performed using a 14 to 18 gauge sampling needle.[86,106] In the blind biopsy procedure the patient is sedated or anesthetized and placed in dorsal or right lateral recumbency.[106] A stab incision is made mid-way between the tip of the xiphoid notch and the left costal margin[106]. The needle is placed through the incision and advanced cranially at a 20° to 30°.[106] After the tip of the tru-cut needle has passed through the liver capsule, the instrument is deployed and then

removed.[106] When the liver lacks a focal lesion, a sample is typically obtained from the left lateral liver lobe because the left lateral lobe is large and easily accessible, as well as relatively distant from large biliary and vascular structures.[30] The needle biopsy procedure may not be appropriate for small patients or those with a small liver where the length of the needle exceeds the depth of the liver.

In addition to being less invasive, needle biopsy of the liver can be performed quickly, and at considerably less cost than other biopsy methods. The tru-cut needle has the benefit of being sharp enough to sample fibrotic liver tissue that may be difficult to obtain with other methods such as aspiration needles.[30,84] Additionally, tru-cut needle biopsies obtain deep tissue samples from well beneath the liver capsule, avoiding histopathologic artifacts that peripheral samples can contain.[107] Finally, due to the minimally invasive nature of percutaneous needle biopsy it is associated with minimal patient morbidity.[108-110]

Unfortunately, the accuracy of histopathologic interpretation of percutaneous needle biopsies of the liver has been questioned.[86] Biopsy samples obtained using 18 and 16 gauge needles are consistently small and may be of insufficient quality to allow an accurate diagnosis.[85,86,108] This is illustrated by a study finding 48% agreement in histopathologic diagnosis between 18 gauge needle biopsies and surgical wedge samples taken from the same animal.[86] The needle biopsies in this study also showed significantly more severe inflammation compared to surgical samples.[86] Another shortcoming of small needle biopsies is that they are prone to fragmentation which may distort histopathologic structure, and in one report 68% of 18 gauge samples were

fragmented into 3 or more pieces.[86,104] In human medicine, the minimum number of portal triads for a liver biopsy sample to be considered diagnostic quality is 6-8.[109] Although no minimum recommended triad number has been determined for dogs, needle biopsy specimens in this species routinely produced samples with <6 portal triads.[86] Small needle biopsies may result in inaccurate results due to the lack of lobular architecture in the sample.[104] Eighteen gauge needle biopsies frequently contain portions of approximately 12 hepatic lobular structures.[104] Zonal pattern, multifocal disease, and bridging fibrosis are much more difficult to identify without complete lobular architecture intact.[104]

Unfortunately, the previous reports[85,86,108] addressing the limitations of needle biopsies employed relatively thin needles (18 and 16 gauge) when the current recommendation for biopsy needle size in the average-sized dog is 14 gauge.[30] Therefore, it remains to be determined if the limitations of histopathologic interpretation of the liver obtained using small gauge needles can be overcome using a larger instrument.

Laparoscopic biopsy

Laparoscopic liver biopsy has become an increasingly utilized minimally invasive alternative to surgical liver biopsy.[111,112] For this procedure the patient is placed under general anesthesia and following aseptic preparation of the abdomen, a Veress needle is introduced into the abdomen for gas insufflation.[112] Carbon dioxide is typically chosen for insufflation because of its safety in preventing air emboli and spark ignition during cauterization.[111] After insufflation, working instruments can be introduced into the abdomen through a trochar-cannula unit.[111] Laparoscopic liver biopsies can be collected

from either the edge or the center surface of a liver lobe, and multiple samples may be obtained from multiple lobes.[113]

Laparoscopic liver biopsy using laparoscopic cup biopsy forceps routinely provides liver samples with greater than 6-8 portal-triads.[85,114] A study in humans has shown that the large biopsies obtained laparoscopically are superior to blind needle biopsy for diagnosing cirrhosis.[115] Additionally, laparoscopy allows for visual inspection of the surface of the liver[85] and can identify hepatic lesions as small as 0.5 cm, which may be difficult to detect by other minimally invasive methods. Furthermore, if excessive bleeding is noted after biopsy acquisition, laparoscopic hemostatic intervention may be employed.[113] Finally, laparoscopy has a low complication rate and a short recovery time for the patient.[113,114]

Despite the advantages of laparoscopic biopsy vs. needle biopsy, laparoscopy requires specialized equipment and advanced training for proper use. Laparoscopy may be difficult or unsafe to perform in very small patients due to the size of the instruments used. Additionally, the procedure requires general anesthesia which carries risks and may not be appropriate for severely ill or debilitated patients. Finally, laparoscopic liver biopsy is expensive compared to needle biopsy or liver aspirate and may be cost prohibitive for some pet owners.

Surgical liver biopsy

Currently, the gold standard for obtaining a liver biopsy in the dog is a surgical biopsy via celiotomy.[86] While many techniques for surgical liver biopsies have been described,

the most commonly employed techniques are the suture fracture technique, wedge biopsy, and punch biopsy techniques. Surgical liver biopsies allow large samples to be collected from various lobes and surgical biopsy samples have been shown to typically contain greater than 8 portal triads.[85,86] Furthermore, surgery allows visual inspection and palpation of hepatic tissue to identify lesions that may otherwise not be apparent. With celiotomy the biopsy site may be monitored for excessive bleeding and the surgeon can intervene if necessary. Finally, the surgical biopsy may be performed on any size patient, where the less invasive techniques may not be appropriate for very small patients.

Unfortunately, abdominal surgery is an expensive and invasive procedure with considerable patient morbidity and recovery time. Celiotomy requires general anesthesia which may not be appropriate for severely debilitated patients. Furthermore, the accuracy of surgical biopsy obtained from the periphery of lobes has been questioned due to artifacts present in the liver capsule that are not in the rest of the liver.[107,116,117] It has been reported in humans that 55% of wedge biopsies from the edge of the liver showed pathologic changes not present in deeper liver samples.[107] Finally, some have suggested that abdominal surgery itself may actually alter hepatic histopathology due to prolonged exposure from the abdomen or handling of the liver tissue.[118] These limitations have not been explored in dogs, and leave the question of what the best biopsy technique of the canine liver should be.

e. Complications of liver biopsy

It is important that the clinician consider the possible risk versus benefit for each patient prior to recommending biopsy of the liver. While the risk associated with liver biopsy is

low[89,108-110] there is always the potential of life threatening complications.[119-122] Approximately 1 to 3 percent of human patients require hospitalization for complications after a liver biopsy.[109] Surgical, laparoscopic, and sometimes needle biopsy procedures require general anesthesia with associated anesthetic risks.[123,124] Additionally, the liver's rich vascular supply combined with the hemostatic abnormalities associated with liver disease makes hemorrhage the most serious complication of a liver biopsy.[22,85,89] Both surgical and laparoscopic biopsy techniques allow for immediate intervention should excessive hemorrhage from the biopsy site occur, but not with percutaneous needle biopsy.

In humans, bleeding complications after percutaneous needle biopsy are reported to occur in 0.06% to 1.7% of cases.[125] Reported mortality rates are between 0.009% and 0.33% [125] with death usually resulting from hemoperitoneum.[88] Rates of hemorrhage and mortality are greatest among patients who undergo biopsy of malignant lesions,[88,109] and a greater incidence of bleeding after biopsy has been observed with tru-cut biopsy needles as compared to other types of percutaneous needles. However, complication rates have decreased through the use of sonographic guidance.[126]

Percutaneous biopsy of the canine liver has been reported to have a similarly low complication rate; between 0% and 1.7%.[89,127] Again, hemorrhage is the most commonly reported complication, although biliary rupture necessitating surgical repair has also been reported. The higher prevalence of complications with tru-cut needles reported in humans has not been investigated in dogs.

The severity of liver disease or coagulation abnormalities does not correlate well with the risk of complications after percutaneous biopsy.[22,89] Therefore, it is difficult to predict which patients will experience complications after the procedure. Thus, the least invasive method known to produce accurate results should be chosen.

C. Quality of liver sample

a. Accuracy of histopathology

To accurately diagnose liver disease, a liver biopsy must be of adequate size and quality to ensure reliable histopathologic evaluation.[85,86,109,128,129] A clinician must know that the specimen obtained is an adequate representation of the overall disease process in the liver. In humans, the minimum standard for size of a liver needle biopsy is at least 1.5 cm in length with at least 6-8 portal triads.[109] Samples that contain less than 6-8 portal triads, or samples with multiple fractures are considered inadequate because they may produce inaccurate results.[86] Small samples are not ideal because of the lack of hepatic lobular architecture, which precludes interpretation of zonal patterns, bridging fibrosis.[104] Additionally, larger biopsies increase the likelihood of detecting multifocal disease.[104]

Several studies in humans have scrutinized this recommendation for size of liver biopsies and suggested if a larger biopsy may be needed to accurately diagnose and stage inflammatory hepatopathies. Holund et al[130] reported that needle liver biopsy samples 0.5 cm long or longer were adequate for diagnosis of acute hepatitis but inadequate for diagnosing chronic hepatitis or cirrhosis. A later study[131] concluded that a 1.5 cm, 16 gauge specimen was necessary for accurate diagnosis of chronic hepatitis. However,

another report comparing biopsy specimens of 0.5 cm, 1 cm, 1.5cm, and >2 cm in length concluded that a 1 cm liver sample was adequate for diagnosis of necroinflammatory activity and fibrosis.[132] When Colloredo et al[129] compared percutaneous biopsies of various lengths, they concluded that a biopsy sample should be a minimum of 2 cm in length and contain at least 11 portal triads for reliable grading and staging in chronic viral hepatitis. Additionally, in patients with chronic viral hepatitis, thin-needle biopsies (21 gauge) have been shown to produce specimens with lower scores for fibrosis, necrosis, and inflammation compared with larger (17 gauge) biopsies.[133]

In dogs the minimum number of portal triads required to allow accurate histopathologic interpretation is unknown. However, one study demonstrated only a 48% agreement in histopathologic diagnosis between small samples containing less than 6 portal triads and larger surgical samples taken from the same animal, implying that samples with less than 6 portal triads are inaccurate.[86] This study also reported needle biopsies had higher inflammation scores when compared to surgical samples, which is in contrast to human studies in which small needle biopsies produced samples with less inflammation compared to larger samples.[86]

Small needle biopsies may produce inaccurate results due to the lack of complete lobular architecture in the sample.[104] Evaluation of partial lobular structures precludes interpretation of zonal patterns which may support a specific etiology or disease. For example, hepatotoxins may result in hepatocellular swelling, steatosis, and necrosis with a centrilobular, periportal, midzonal, or massive pattern.[30] The pattern as well as the severity of changes may be supportive of a specific toxic agent. Small samples limit the

ability to distinguish between histologically similar lesions such as well differentiated hepatocellular carcinomas and hepatocellular adenomas.[30,134] Furthermore, small samples make it more difficult to assess the severity of lesions. For example, differentiating cirrhosis from fibrosis requires identification of fibrosis, nodule formation, and portal-central vascular anastomosis.[30] Small samples with incomplete architecture may show only a portion of the lesions, making proper diagnosis difficult. In humans, 20% of patients with cirrhosis were misclassified as having less severe disease based on blind percutaneous biopsies compared to larger laparoscopic biopsies.[115]

However, the results of studies in humans that generate biopsy recommendations may not be applicable to dogs as the goals of liver biopsy are typically different between human and veterinary medicine. For example, the etiology of a human patient's hepatopathy is likely to be diagnosed or highly suspected prior to the biopsy procedure based on previous laboratory findings and imaging studies. Biopsy may be pursued for confirmation of disease, or staging once treatment has been initiated. The size and frequency of biopsy procedures can be chosen based on the etiology. In contrast, the etiology of many canine hepatopathies is generally unknown prior to biopsy, with multiple possible differential diagnoses. This could suggest the ideal biopsy in dogs may need to be larger than what are recommended for human medicine.

Measurement of micro-minerals

The distribution and concentration of copper in the liver is of particular importance, as several dog breeds are predisposed to familial hepatic copper accumulation.[135-139] In Bedlington terriers, an inherited genetic mutation in the COMMD1 gene results in

defective hepatocellular copper excretion.[38,140] Copper accumulation in affected dogs begins in centrilobular (zone 3) hepatocytes and progressively leads to hepatocellular inflammation and necrosis.[139,141] This is in contrast to copper accumulation secondary to chronic inflammation which typically begins in the periportal regions (zone 1). Small biopsy samples lacking complete lobular structures limit the ability to correctly identify centrilobular copper accumulation; therefore, biopsy samples large enough to identify intact lobular architecture are critical for the correct diagnosis.

Copper, iron, and zinc levels are frequently measured in hepatic tissue biopsy samples to aid in identification of disease and guide therapeutic decisions.[136,142-147] In humans a minimum of 3 mg to 5 mg of dry-weight tissue (approximately 6-10 mg of wet-weight tissue) is the minimum standard required for micro-mineral analysis.[148] Smaller biopsy samples like those obtained from a needle biopsy have been shown to produce inaccurate copper concentration results in several species, presumably due to variability in copper concentration throughout the hepatic parenchyma..[149-151] For example, hepatic regenerative nodules may contain variable (more or less) copper levels when compared to adjacent hepatic parenchyma.[135,150] Similar to studies in humans, 18 gauge needle biopsy samples contained significantly lower copper and iron concentrations compared with surgical wedge biopsy samples in dogs.[128] This report concluded that a biopsy sample of 12 mg of wet weight tissue is adequate for metal analysis in the dog.[128] However, the accuracy of results for mineral analyses from larger needle biopsy samples is still unknown.

b. Distribution of lesions within hepatic tissue

Because many liver diseases result in focal or multifocal lesions, a potential limitation of the percutaneous needle biopsy is the possibility of sampling error from focal sampling of non-homogenous liver disease. When using ultrasound guidance in a dog with presumed diffuse disease, the left lateral lobe of the liver is usually biopsied.[30] For this sample to be representative of the liver as a whole, diffuse hepatic diseases must produce histopathologic lesions distributed evenly throughout all hepatic lobes. If this occurs, sampling the left lateral lobe will be representative of overall hepatic disease. In several conditions in humans, including nutritional fatty liver disease,[152] sarcoidosis,[153] and brucellosis, the changes are diffuse and reflected in a focal biopsy.[154] Unfortunately, similar information is not available in dogs.

However, in focal, multifocal, or even some diffuse diseases, a single needle biopsy may not provide a representative sample that reflects the primary disease process. As previously discussed, hepatic copper accumulation is an example of diffuse change that is not evenly distributed throughout the liver. Humans with copper storage disease have been shown to accumulate copper preferentially in the right rather than the left liver lobe.[150] In contrast, studies addressing copper concentrations in the liver of newborn humans have reported that copper tends to be preferentially stored in the left lobe more than in the right.[151] Therefore, sampling of only one lobe may not accurately reflect the overall concentration of copper in the liver. Similarly, great variation in liver glycogen concentration has been demonstrated in the livers of rabbits.[155] Differences in glycogen concentrations between lobes[156] as well as marked variation in glycogen between samples from the same lobe have been reported.[155]

When needle biopsy and samples obtained at autopsy from human patients were compared, needle liver biopsy was accurate for identification of fatty infiltration, hepatocellular necrosis, leukemic infiltrate, and venous congestion.[157] However, there was variability in the diagnosis of cirrhosis, chronic active hepatitis, and multifocal diseases such as metastatic carcinoma, and granulomas. Unfortunately, sonographic evaluation of the liver was not utilized in this study, which may have increased the accuracy of needle biopsy for focal or multifocal disease. Maharaj et al[158] similarly evaluated disease variability by collecting three sequential needle biopsies by redirecting the biopsy needle through a single entry site. In this series only 50.7% of patients had uniform findings between all three samples. In that same study the finding of cirrhosis, hepatocellular carcinoma, metastatic carcinoma, or hepatic granulomas were found in all three samples in only 50%, 54.5%, 50%, and 18.8% of patients respectively.[158] These findings clearly illustrate the potential for non-uniform change in hepatic disease.

Conflicting reports exist in humans about the accuracy of needle biopsy for the detection of fibrosis and inflammation. One report identified as much as a 10% variation in the findings of fibrosis and inflammation between needle biopsy samples from the same liver.[159] A study of humans with primary biliary cirrhosis revealed considerable variation in fibrous tissue, with only 20% of patients having a consistent degree of fibrosis throughout all lobes.[160] Furthermore, an investigation of the hepatic pathology in patients with hepatitis C reported that 33.1% of samples showed significant variation between the left and right liver lobes in the same patient.[161]

In contrast, a study comparing percutaneous liver biopsies obtained from both the left and right liver lobes in patients with hepatitis C virus showed no significant difference between grade and stage of disease between the two specimens.[162] Additionally, a post-mortem study of human livers affected with various stages of hepatitis showed diffuse anatomical lesions throughout all liver sections.[163] Another report of 22 livers with diffuse disease found that needle biopsy specimens correlated with autopsy findings in all cases suggesting an even distribution of disease.[164] In contrast, comparisons of laparoscopic guided needle biopsies of the right and left lobes of humans with chronic liver disease revealed that 23.5% of samples had histologic differences between the right and left lobes.[165] Of the samples that showed differences, 35% actually had a different diagnosis in each liver lobe.[165] Additionally, multiple studies comparing the right and left liver lobes of humans with nonalcoholic fatty liver disease have shown variation in disease stage[166] and necroinflammatory activity.[167,168] Together, these reports suggest that substantial variation between adjacent regions of the liver can exist in a single patient.

Finally, studies in humans have shown that histologic artifacts may be found in the liver capsule and subcapsular region that are not diffuse throughout the liver. Biopsy of superficial tissue or from the margin of a liver lobe may contain artifacts such as fibrosis, increased stroma, or a nodular change extending 0.2 cm – 0.5 cm below the capsule.[107,117] A study comparing superficial wedge biopsies and deep needle biopsies form the same patients reported increased fibrosis in wedge samples, which they attributed to subcapsular artifacts.[116]

Less is known about the variation in disease distribution throughout the canine liver. When liver needle biopsies were compared to necropsy specimens in dogs, there was a 53% agreement between samples.[169] All needle samples were collected blindly from the left side, but the size of the biopsy instrument was not reported, nor was the quality of the samples. Petre et al[114] found in dogs that 14% of laparoscopic biopsies obtained from different liver lobes had different diagnoses. In that study, two cases had a different type of hepatic disease identified between samples, such as vacuolar hepatopathy versus fibrosis. In two cases neoplasia was identified in at least one sample but not in the others, and in three cases at least one biopsy sample was diagnosed as normal when disease was present in the other samples. Based on these data the authors concluded that multiple biopsies should be collected to ensure accurate results. It is likely that canine hepatopathies thought to be diffuse disease may have an uneven distribution of histologic changes. With regards to canine hepatic micromineral distribution, marked variation in copper concentration was found when needle biopsy samples were compared to wedge samples.[128] The difference was attributed to variation in copper content throughout lobes. However, the copper concentrations in all samples were elevated, and results in each biopsy would not have changed the clinical severity score of the patient.[128] The other dogs evaluated in this study showed no significant difference in copper concentration between needle and wedge samples.[128]

Further investigation into histologic variability in the canine liver has likely been impeded by the lack of information about the etiology of canine liver disease. Reports in humans typically focus on patients with a specific diagnosis such as hepatitis C or non-alcoholic steatohepatitis, and studies are aimed to define the best biopsy method for that

specific disorder. However, canine liver disease, and in particular diffuse liver disease, is less well-defined, and therefore canine studies frequently assess multiple categories of liver disease together. If lesion distribution within the canine liver varies depending on the disease, different biopsy methods should ideally be based on the category of disease. Further research is needed to determine if there is one ideal biopsy method or if biopsy requirements vary with disease etiology in the dog.

D. Current percutaneous liver biopsy standards

a. Recommendations in human medicine

Percutaneous needle biopsy and transjugular biopsy are the two techniques most frequently employed.[170] Percutaneous needles are considered large when the external needle diameter is > 1mm (14 gauge to 19 gauge) and small when the diameter is <1 mm (20 gauge or greater).[170] For percutaneous biopsy, tru-cut needles and suction Menghini biopsy needles are used most often.[170]

Historically, the accepted minimum standard for the size of a percutaneous liver biopsy was 6-8 portal triads.[109] However, several recent studies have suggested that larger biopsy samples are needed to accurately diagnose the degree of fibrosis and inflammation, particularly in patients with hepatitis C virus.[129-133,171] The newly proposed minimum standard for a percutaneous biopsy sample for staging of hepatitis C is that it should be at least 2.0-2.5 cm in length and contain at least 11 portal triads.[129,170] However, to obtain samples that meet these criteria, more than one percutaneous sample is frequently required which increases the possibility of biopsy complications. Due to

increased risk to the patient, some authors feel it is unrealistic to obtain biopsies that fulfill these criteria and have called for further studies.[170]

b. Recommendations in veterinary medicine

Ultrasound guided percutaneous biopsy with a tru-cut biopsy needle is the most frequently employed non-invasive method of liver biopsy in veterinary medicine. Needles ranging from 14-18 gauge are used. However, many[30,84-86] question the accuracy of histopathology results from small gauge (less than 16 gauge) samples.

The World Small Animal Veterinary Association (WSAVA) Liver Standardization Group is a collaboration of veterinary specialists who are experts in the field of hepatology. The WSAVA has published a consensus statement defining the histopathologic criteria of canine liver diseases along with recommendations for standards of biopsy acquisition. For collection of a tru-cut needle biopsy the WSAVA recommends that at least two to three good samples are submitted for histopathology. Each sample should be unfragmented and at least 1-2 cm in length. Fourteen gauge needle samples are recommended in dogs with 16 gauge needles reserved for very small dogs.[30] The amount of tissue required for quantitative copper analysis varies from 50 mg to 5 g depending on the laboratory.[172,173] The Colorado State University Veterinary Diagnostics Laboratory recommends submission of 5 grams of tissue or three 1cm long needle biopsies from a 14 gauge needle.[173]

E. Summary

The ideal biopsy technique and biopsy size are unknown in veterinary medicine. Until the ideal method of liver biopsy is identified, dogs may be subjected to inaccurate invasive testing. Aim 1 of this study is to identify the method of liver biopsy which most consistently produces adequate samples and accurate histopathology results. Specifically, we will test the hypothesis that liver biopsy specimens obtained via surgical punch, laparoscopic cup biopsy, and percutaneous 14gauge tru-cut biopsy will result in similar histopathologic diagnoses to those from deep sectioned samples of liver obtained at necropsy. Aim 2 of this study is to evaluate the distribution of histologic changes throughout the canine liver by comparing samples obtained from each liver lobe at necropsy. The hypothesis tested will be that discordant morphologic diagnoses will occur with equal frequency between lobes.

CHAPTER 2: A COMPARISON OF LIVER BIOPSY METHODS IN DOGS.

A. Introduction

Histopathology of the liver provides information about the cause, chronicity, and reversibility of disease.[84,87] However, reliable histopathologic results are dependent upon a liver sample of adequate size and quality.[109,129] In humans, biopsies containing 6 - 11[109,129] portal triads are recommended to ensure accurate interpretation. Samples with few portal triads or those that fracture into multiple pieces are considered inadequate.[109,129,174]In veterinary medicine the minimum number of portal triads necessary for accurate histopathologic interpretation is unknown.

The World Small Animal Veterinary Association (WSAVA) Liver Standardization Group guidelines suggest that needle biopsy is adequate and that surgical liver biopsy is unnecessarily invasive. However, several studies in dogs have questioned the accuracy of needle biopsies.[85,86,169] When needle biopsies were compared to necropsy specimens in dogs, there was a 53% agreement between samples.[169] However, the size of the biopsy instrument was not reported, nor was the quality of the samples. Additionally, one study demonstrated only a 48% agreement in histopathologic diagnosis between 18 gauge needle biopsies and surgical samples taken from the same animal.[86] Finally, punch and cup liver biopsies were shown to routinely produce samples with greater than 6-8 portal triads[85], while 18 gauge and 16 gauge needle biopsy samples produced fewer than 6 portal triads.[85,86]

Liver biopsy is an invasive procedure that is associated with some risk. Hemorrhage from the biopsy site is usually minimal but can be a potentially life threatening complication of any type of liver biopsy.[85,106,120,121] Because different methods of liver biopsy have dissimilar risks, morbidity, and cost, it is important to identify the biopsy technique that results in an accurate diagnosis with the least potential to harm the patient.

Currently the WSAVA Liver Standardization Group recommends 14 gauge needle samples in most dogs, with 16 gauge needles reserved for small patients. The adequacy of samples obtained by this method is unknown as previous studies have evaluated smaller biopsy needles. Therefore the primary goal of this study was to compare liver biopsy samples collected by punch, cup, and 14 gauge needles to identify the method that most consistently produces samples that reflect the histopathology of the liver. We hypothesized that liver biopsy specimens obtained via punch biopsy, cup biopsy, and 14 gauge needle biopsy would result in similar histopathologic diagnoses to those found on deeply sectioned samples of liver obtained at necropsy.

B. Materials and Methods

a. Experimental protocol

The study was approved by the Institutional Animal Care and Use Committee of Virginia Tech. This was a prospective study of dogs presented to the necropsy service at the Virginia-Maryland Regional College of Veterinary Medicine, Veterinary Teaching Hospital (VTH). All dogs were patients of the VTH who died or were euthanized. All samples were collected within three hours of death and by the same investigator (SK).

Biopsy samples collected from each cadaver included an 8 mm punch biopsy,[a] a 5 mm cup biopsy,[b] and a 14 gauge biopsy needle sample.[c] All techniques were performed in a manner that simulated collection in a clinical patient as closely as possible. The punch biopsy was collected by advancing the cutting edge of a biopsy punch[a] at a ninety degree angle into the surface of the liver parenchyma near the center of the left lateral lobe. The cup biopsy was collected by advancing the open jaws of the cup biopsy forceps[b] at a ninety degree angle into the surface of the liver parenchyma near the center of the left lateral lobe. The needle biopsy was collected with a semiautomatic 14G biopsy needle[c] by advancing the needle into the center of the left lateral liver lobe at a ninety degree angle to the surface.

Biopsies using each technique were collected until a non-fractured specimen that completely filled the sampling channel of the instrument was obtained. After biopsy acquisition, two deep tissue samples of at least 2 cm x 2 cm x 1 cm were taken from the left lateral lobe. These large samples (designated "necropsy" samples in this manuscript) were used as the standard for morphologic diagnosis. If a focal liver lesion was noted (e.g., mass or discoloration), the procedures for obtaining biopsy samples and large tissue samples were repeated at the lesion site. Samples were obtained through a midline abdominal incision, and all samples were taken from the left lateral liver lobe in order to simulate sample collection during percutaneous ultrasound-guided needle biopsy.[30] All biopsy specimens were taken within 5 cm to minimize hepatocellular variation within the lobe.

All tissue samples were immediately fixed in neutral-buffered 10% formalin. After fixation, samples were arranged in paraffin cassettes for embedding and processing. Five micron thick sections were prepared and stained with hematoxylin and eosin. Two-hundred eighty four slides from 71 sample sites were randomized and evaluated by a board certified veterinary pathologist (KZ), who was unaware of their hospital case identity, for standardized evaluation as described below.

All samples were assigned a score in each of 16 histologic criteria[86]: hepatocellular atrophy, hepatocellular hypertrophy, biliary hyperplasia, ceroid lipofuscin accumulation, hemosiderin accumulation, canalicular cholestasis, congestion, extramedullary hematopoiesis, vacuolar change, fibrosis, tissue inflammation, lobular collapse, hepatocellular necrosis, neoplasia, thrombosis, and vascular abnormalities. Scores were on a scale of 0-3 with 0 representing no change and 3 representing severe change. Neoplasia was assessed as present or absent.

Hepatocellular atrophy was identified by cords being closer together, small hepatocytes, increased numbers of portal triads in a given area, and a wrinkled capsule.[175] Hepatocellular hypertrophy was defined by the presence of hepatocytes of increased size and increased cytoplasmic basophilia.[175] Biliary hyperplasia was scored on the basis of number of small biliary duct profiles located within the portal triad areas.[175] Ceroid lipofuscin was defined as a lightly golden-yellow, granular to globular, hepatocellular cytoplasmic pigment.[175] Hemosiderin accumulation was defined as a brown crystalline pigment within both hepatocytes and Kupffer cells.[175] Canalicular cholestasis was scored based on the identification of green bile plugs within the bile canaliculi.[175] Congestion

was diagnosed based on distention of hepatic sinusoids by erythrocytes.[175] Extramedullary hematopoiesis was diagnosed when foci of hematopoietic precursors cells were identified within the biopsy.[175] Vacuolar change was identified based on the presence of swollen hepatocytes with cytoplasmic vacuoles that were either distinct or indistinct, and, either single or multiple, as well as those with finely reticulated cytosol.[175] Fibrosis was diagnosed by a proliferation of fibroblasts and collagen appreciable by hematoxylin and eosin stain.[175] Tissue inflammation was classified as acute hepatitis, chronic hepatitis, reactive hepatitis, and cholangiohepatitis. Acute hepatitis was characterized as a combination of inflammatory cells with neutrophils in majority, hepatocellular apoptosis and necrosis, with or without regeneration.[34] Chronic hepatitis was characterized by a combination of hepatocellular apoptosis or necrosis with predominance of lymphocytes, plasma cells and macrophageswith or without a smaller neutrophilic component, regeneration and fibrosis.[34] Reactive hepatitis was characterized by neutrophilic or mixed inflammation in portal areas and the hepatic parenchyma without necrosis.[34] Cholangiohepatitis was characterized by neutrophilic, lymphocytic or mixed inflammation involving portal region hepatocytes as well as bile ducts.[34] Hepatocellular apoptosis was characterized by shrunken hepatocytes, with eosinophilic cytoplasm, and condensed nuclei surrounded by an empty halo.[34] Lobular collapse was diagnosed by loss of normal lobular architecture due to loss of hepatocytes.[175] Hepatocellular necrosis was diagnosed by the presence of swollen cells, with eosinophilic cytoplasm, and fragmented or pyknotic nuclei.[34] Neoplasia was diagnosed by identification of atypical, dysplastic either metastatic or hepatic cells within the biopsy specimen.[175] Thrombosis was identified by the presence of thrombi within hepatic

vasculature.[175] Vascular abnormalities were scored based on identification of small or absent portal veins, arteriolar proliferation, with or without hepatocellular atrophy.[175] The criteria scores of the two necropsy samples were averaged and served as the standard to which the other samples were compared.

Based on the histologic criteria scores, a morphological diagnosis was assigned to each of the 4 specimens (three biopsy methods and deep necropsy sample) based on the World Small Animal Veterinary Association (WSAVA) Liver Standardization Group guidelines.[30] To be conservative, only histologic criteria scores ≥ 2 were considered as part of the final morphologic diagnosis. The morphologic diagnosis assigned to the necropsy samples was considered the definitive diagnosis. If the morphologic diagnoses from the two necropsy samples did not agree, all specimens from that dog were censored from analysis. Finally, the number of portal triads present in each sample was recorded. The basis for identification of a portal triad was the presence all triad structures (hepatic artery, portal vein, and bile duct).

b. Statistical Analysis

Agreement between definitive morphologic diagnosis and the morphologic diagnosis of the biopsy specimens were assessed by calculating kappa coefficients. The sensitivity and specificity of each biopsy type as compared to the necropsy samples was calculated. The proportions of concordant biopsy results were compared with logistic generalized estimating equations (GEE) analysis. The mean number of portal triads between sample types was compared with a mixed model ANOVA. The mean score for each of the 16 histologic features was calculated for all samples of each biopsy type. These mean scores

for each histologic feature were compared using a linear GEE analysis to detect significant differences in the histologic characteristics between biopsy samples and standard necropsy samples. All analyses were performed using commercial software.[d] Significance was determined at $P<0.05$

C. Results

Seventy dogs and 71 total sample sites (one dog had a focal lesion) were included in this study. No cases were censored due to disagreement between the 2 necropsy samples. Morphologic diagnoses in the necropsy samples were no abnormality (18/71; 25.4%), vacuolar hepatopathy (18/71; 25.4%), neoplasia (8/71; 11.3%), primary fibrosis (6/71; 8.45%), chronic hepatitis (5/71; 7.0%), congestion (5/71; 7.0%), chirrosis (5/71; 7.0%), necrosis (3/71; 4.2%), cholangiohepatitis (1/71; 1.4%), reactive hepatitis (1/71; 1.4%), and cholestasis (1/71; 1.4%).

There were no significant differences ($P = 0.29$) in the proportion of discordant samples between biopsy types (Table 1). Cohen's kappa coefficient for the needle, cup, and punch biopsies were 0.59, 0.52, and 0.62 respectively.

The mean number and 95% confidence interval of portal triads in each sampling method was 2.9 (2.58 - 3.2) in needle biopsies, 3.4 (2.7 – 4.23) in cup biopsies, 12.02 (10.31 – 13.73) in punch biopsies, and 30.3 (26.6 – 33.95) in the necropsy samples. Punch biopsies had significantly more portal triads than either cup or needle samples ($P < 0.001$) which were not statistically different from each other ($P = 0.98$). The necropsy samples had significantly more portal triads than the test samples ($P= <0.0001$). The number of

portal triads could not be reported in 8 needle samples, 11 cup samples, 12 punch samples and 13 standard necropsy samples due to loss of normal heaptic architecture. In the 13 necropsy samples where portal triads could not be reported, the final diganosis was neoplasia (5), cirrhosis (4), fibrosis (1), necrosis (1), chronic hepatitis (1), and congestion (1).

The sensitivities of the biopsy methods as compared to the necropsy samples were similar and ranged from 54.7% - 66%. The specificities were also similar between methods and were higher than the sensitivities ranging from 77.7% - 80.3% (Table 1). The sensitivity and specificity of each biopsy method was calculated for each different diagnosis and the highest sensitivities were found in in dogs with vacuolar hepatopathy, normal hepatic histopathology, and neoplasia (Table 2). Results were not reported for necrosis, cholangiohepatitis, reactive hepatitis, or cholestasis due to the small number of cases in each category. While many of the measures of test performance were variable, the negative predictive value was consistently high in all biopsy methods and all morphologic diagnoses.

Diagnoses in the 8 livers with neoplasia included histiocytic sarcoma (3), lymphoma (3), round cell sarcoma (1), and spindle cell sarcoma (1). The sensitivity for diagnosis of neoplasia was 62% for needle biopsies; 75%, for cup biopsies; and 87.5% for punch biopsies. The specificity for neoplasia was 100% in all three biopsy types. Overall, the sensitivity for the diagnosis of fibrosis was low, ranging from 16%-50%. However, the sensitivity of the needle biopsy method was substantially lower than either cup or punch biopsies for fibrosis.

The mean scores for each of the histologic features were compared amongst the biopsy types and several significant differences from the necropsy standard were identified (Table 3) The needle biopsy samples identified significantly less hepatocellular atrophy, biliary hyperplasia, hemosiderin, and congestion compared to the necropsy samples. The cup biopsy samples identified significantly less biliary hyperplasia, hemosiderin, and congestion when compared to the necropsy samples. Finally, the punch biopsy samples showed significantly less hepatocellular hypertrophy and hemosiderin than the necropsy samples. In the 6 cases with a predominant histopathologic abnormality of fibrosis, the mean fibrosis score in the necropsy samples was 2.5 which was significantly higher than 1.5 in the punch (P= 0.014), 1.4 in the cup samples (P= 0.014), and 0.5 in the needle samples (P= <0.001). In the 5 cases of chronic hepatitis the mean inflammation score in the necropsy samples was 2.6 which was significantly higher than 1.5 in the cup samples (P=0.002), and 1.4 in the needle samples (P=<0.001), but not significantly different from 2.1 in the punch samples (P=0.15)

D. Discussion

Results of this study indicate that 14 gauge needle, cup, and punch biopsies of the liver all have similar agreement to larger necropsy samples. This level of agreement is insufficient when a single biopsy is taken by any tested technique. This disparity between the biopsy samples and the necropsy standard seemingly occurs as a result of variable distribution of morphologic features within a liver lobe which might be overcome by obtaining multiple biopsies, a larger single sample, or perhaps biopsies from multiple lobes. The paired large samples from each dog had identical histopathologic

diagnoses, while the smaller samples obtained using the various biopsy methods being studied had less consistent agreement with the large samples. Because all the samples were obtained within 5 cm of each other, the size of the specimen obtained by the biopsy methods was the main factor influencing the histopathologic review and interpretation.

Smaller biopsy samples produced fewer portal triads. The number of portal triads in the needle and cup samples were not different, and both contained fewer than recommended in the human literature.[109,129] Additionally, these numbers are lower than previous reports where 18 gauge needle biopsies had a median of 4 portal triads[86] and 16 gauge needle samples had a mean of 6-7.9 portal triads[85] compared to the mean of 2.9 and median of 3 found in this study. This discrepancy may be attributed to the strict criteria used for identification of portal triads in this study. In the present study, all 3 structures comprising the portal triad had to be clearly identified, while other studies that did not describe their methodology may have counted portal areas without all components of the triad visible. Despite the punch biopsy samples containing significantly more triads than the other biopsy methods, the overall proportion of discordant results was not different. Therefore, when the number of portal triads in samples ranges from 3-12, the final histopathologic interpretation is unlikely to vary. However, because of the relatively poor agreement with the necropsy samples, it is reasonable to assume that biopsies larger than those obtained in this study may enhance the likelihood of a correct diagnosis. Because techniques used to obtain larger biopsies might result in increased risk for hemorrhage, multiple biopsies from different locations of a lobe might be the best method to safely acquire adequate tissue.

Additionally, portal triads could not be reported in 13 (18%) of the necropsy samples due to severe distortion in the hepatic architecture. This raises concern for the use of portal triad numbers as a measure of biopsy quality, as these samples were large but did not contain recognizable triad structures. However, the diagnoses in the majority of these cases were neoplasia or cirrhosis and it is likely that in such severe disease, the size of sample may be more important that the number of portal triads.

The highest sensitivities were found in dogs without hepatic disease, where values ranged from 77% - 83% and specificites ranged from 68% - 81% (Table 2). In dogs without hepatic disease, the sensitivity and positive predictive value of needle biopsy samples was 83% and 53% which compares favorably to previous reports.[86] Additionally, while the sensitivity for detection of hepatic neoplasia was variable, the specificity was 100% in all biopsy methods tested. The sensitivity of needle biopsy for diagnosis of neoplasia in the present study was similar to that of another study (80%) using a smaller instrument.[86] However the majority of neoplasms in our population were systemic, and it is unclear if similar results would be found in dogs with metastatic or multifocal neoplasia.[158]

The lowest sensitivity and positive predictive value was for the detection of fibrosis, especially in the needle samples. There were no significant differences in the mean histologic scores for fibrosis between the needle biopsies and the necropsy samples (Table 3). However, when the six cases with a predominant histologic abnormality of fibrosis were analyzed separately, all three sampling methods had a significantly lower mean fibrosis score than the necropsy samples. In these cases the punch and cup samples had a concordant diagnosis in 3 samples, while needle samples had a concordant

diagnosis in 1 case. These findings suggest that large tissue samples may be necessary to accurately reflect the degree of fibrosis when severe disease is present. This is in contrast to a previous study where smaller needle biopsy samples had higher histologic scores for fibrosis.[86] Our results mirror those of several studies in humans where fibrosis scores declined with smaller biopsy size.[174,176] This discordance in the fibrosis scoring is likely caused by variation in severity of fibrosis throughout or between lobes, which has been documented in humans. In a report of patients with primary biliary fibrosis, whole section scanning of the liver at the time of transplantation revealed that only 20% of these livers had consistent fibrosis throughout the entire organ.[160]

A previous study reported that needle biopsies from dogs had higher scores for inflammation when compared to wedge biopsies from the same patient.[86] In the present study there were no significant differences in the histologic scores for inflammation between the sampling methods. However, when the five cases of chronic hepatitis were analyzed separately, scores for both the cup and needle samples were significantly lower than those of the necropsy samples. The punch and cup samples both had concordant diagnoses in 2 cases and the needle samples had concordant diagnoses in 3 cases. These results suggest that while smaller biopsies may accurately represent the degree of inflammatory infiltrate in some hepatic disease, they may underrepresent the severity of disease when chronic inflammation is present. This finding is similar to a report in humans which demonstrated that shorter needle biopsies produced samples with lower inflammatory scores in patients with hepatitis C virus infection.[129] However, in veterinary medicine it is unknown if the reduced inflammation in the small biopsy samples would result in an alternative clinical diagnosis for the patient.

The high number of discordant samples amongst all biopsy methods may be attributed to non-uniform histopathologic lesions throughout the liver lobe. It is likely that canine hepatopathies thought to be diffuse may have an uneven distribution of histologic changes. For example, marked variation in copper concentration was found when needle biopsy samples were compared to wedge samples in dogs.[128] Further investigation into histopathologic variability in the liver has likely been impeded by the lack of information about the etiology of liver disease. Reports in humans typically focus on patients with a specific diagnosis such as hepatitis C virus or non-alcoholic steatohepatitis, and studies are aimed to define the best biopsy method for that specific disease. However, canine liver diseases, and in particular diffuse liver diseases, are less well-defined, and therefore canine studies that assess biopsy methods frequently assess multiple types of liver disease simultaneously. If lesion distribution within the canine liver varies depending on the disease, the recommended biopsy method and number of samples needed should ideally be tailored to the underlying disease. While the most common indication for biopsy is the diagnosis of liver disease, there are situations in which biopsy is recommended to assess circumstances where a biopsy may be obtained to assess prognosis or monitor response to treatment. In these cases the biopsy technique should be based on the disease present.

One limitation of this study is reliance on a single pathologist for interpretation of all of the liver samples. However use of a single pathologist likely resulted in more consistent results between cases, compared with multiple observers.[177] Dogs enrolled in the study were not selected because of known hepatic disease, thus were not representative of the clinical population in which biopsies would be obtained. The influence that a higher prevalence of hepatic disease, would have had on the results of this study is unclear. The

biopsy techniques were performed in a manner that mimicked their antemortem use, but repeated sampling was attempted until a sufficient sample was retrieved. This implies that biopsies obtained in less ideal circumstances might be of lower quality. In a clinical setting acquisition of ideal samples is not always possible, and repeated sampling might put a patient at risk for complications such as hemorrhage. Additionally, in the authors experience needle or cup biopsy of a severely fibrotic liver can be very challenging and may result in small samples. All of this may suggest that in a clinical setting the accuracy of non-surgical biopsies may be even lower than what is reported here. Finally, special stains for highlighting fibrosis may have enhanced evaluation of fibrosis in this study. However, the authors feel that fibrosis was reliably identified by the H&E stain alone and criteria for the identification of fibrosis were uniformly applied amongst the various biopsy types. The use of special stains would likely have resulted in overall higher fibrosis criteria scores but no relative change scores between the biopsy types.

The results of this demonstrate shortcommings in the accuracy of any single liver biopsy by the tested techniques, and suggest that obtaining multiple samples from throughout the liver lobe may be of greater importance than the method of biopsy.

CHAPTER 3: HISTOPATHOLOGIC VARIATION BETWEEN LIVER LOBES IN DOGS.

A. Introduction

Liver biopsy is integral in the diagnosis and management of canine liver disease.[30,84] To accurately diagnose liver diseases, the biopsy specimen must be a reliable representation of the abnormalities within the hepatic parenchyma. Because biopsies collect only a small portion of tissue, there is potential for sampling error associated with non-homogenous distribution of disease. Several reports of human patients suggest that even "diffuse" hepatopathies may result in an uneven distribution of histologic changes.[149-151,158,160,166] For example, studies of human patients with chronic liver disease have shown that disease stage,[166] necroinflammatory activity,[167,168] and even diagnosis can vary between liver lobes.[165]

Although it has been suggested that liver biopsies in the dog should be collected from more than one lobe,[84] little information is available regarding the distribution of histopathologic changes in the canine liver. Petre et. al[114] found that 14% of laparoscopic biopsies obtained from different liver lobes of dogs had discrepant diagnoses. In that study, neoplasia, fibrosis, and other diseases were found in some specimens but not others from the same dog. These results question the reliability of biopsies obtained from only one liver lobe, and suggest that multiple lobes may need to be sampled to obtain an accurate representation.

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 between liver lobes.

B. Materials and Methods

a. Experimental protocol

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 that died or were euthanized and submitted for necropsy. In this prospective observational study, a ventral midline incision was made and a single deep tissue sample of approximately 2cm x 2cm x 1cm was collected by sharp dissection from near the center of each of the 6 liver lobes within three 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 evaluated by a single board certified veterinary pathologist (KZ) who was unaware of the identity of each specimen. A morphological diagnosis was assigned to each sample from 18 categories: no abnormality, neoplasia, cholangiohepatitis, reactive hepatitis, acute hepatitis, chronic hepatitis, necrosis, vacuolar degeneration, congestion, hypoperfusion, thrombosis, lymphatic obstruction, cholestasis, nodular regeneration, nodular hyperplasia, cirrhosis, fibrosis, and hepatocellular atrophy. A single morphological diagnosis was assigned to each specimen based on the predominant pathologic process, and following the World

Small Animal Veterinary Association (WSAVA) Liver Standardization Group guidelines.[30] If more than one disease process was present at more than a minor degree, the dog was excluded from data analysis.

b. Statistical analysis

A logistic generalized estimating equations (GEE) analysis was used to detect differences in the number of discordant results amongst liver lobes from each dog. All analyses were performed using commercial software.[d] Significance was set at a $P < 0.05$.

C. Results

A total of 420 liver lobe samples from 70 dogs were available for review. One case was excluded from the study because more than one disease process was present within a sample. Multiple histological liver diagnoses were found in 30/69 (43.5%) dogs, while the same diagnosis was made in all liver lobes in 39/69 (56.5%) dogs (Table 4). The same diagnosis was present in 6/6 lobes in 39 (56.5%) cases, 5/6 lobes in 10 (14.5%) cases, 4/6 lobes in 10 (14.5%) cases, 3/6 lobes in 7 (10.1%) cases, and 2/6 in 3 (4.3%) cases. The diagnoses that were the most commonly present in all lobes included vacuolar change, neoplasia, and cholestasis, while those least frequently identified in all lobes included chronic hepatitis and fibrosis (Table 4). Ten (14%) of the dogs had ≤ 3 lobes in agreement and therefore did not have a predominant diagnosis. These cases were excluded from the following analysis. Based on the prevalence of lesion distribution in the population studied, biopsy of a single liver lobe would reflect the most prevalent histopathologic diagnosis within the liver in 91.5% of these 59 cases, where a common

diagnosis was present in ≥ 4 lobes. Biopsy of 2 lobes would result in identification of the predominant diagnosis in 98.6% of cases.

The 7 cases of neoplasia included lymphoma (4), histiocytic sarcoma (2), and spindle cell sarcoma (1). Neoplasia was frequently present throughout all lobes, but 2 of the 7 livers with neoplasia did not have the same diagnosis in all lobes. In 1 case of histiocytic sarcoma, the 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 4).

In 11 dogs at least one lobe had fibrosis as the predominant diagnosis. However, fibrosis was rarely a diffuse change, and was only the predominant finding in all 6 lobes in 2 of 11 (18.4%) livers. Fibrosis was identified in only 1 of the 6 liver lobes in 4 of 11 (36.4%) dogs for which fibrosis was the morphologic diagnosis (Table 4).

Twenty dogs had discordant results where 1 or 2 lobes were not in agreement with the diagnosis in the remainder of the lobes. There were no significant differences between any of the liver lobes in the total number of discordant results (P=0.22) (Table 5). The 10 livers in which there were ≤ 3 lobes with the same diagnosis were not included because the predominant disease process could not be determined.

D. Discussion

Results of this study demonstrate variation in the distribution of histopathologic abnormalities between liver lobes. Of the dogs that had a predominant disease process resulting in a diagnosis in ≥4 lobes, biopsy of a single liver lobe would reflect the most prevalent histopathologic diagnosis within the liver in 91.5%, and biopsy of 2 lobes

would result in identification of the predominant diagnosis in 98.6% of cases. These results support the recommendation to obtain biopsy samples from 2 different liver lobes. Because no difference was noted in discordant results between lobes, the lobes chosen for biopsy may be based 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 would be of particular importance in reaching a diagnosis. In addition, the predominant diagnosis could not be determined in 10 (14%) of the dogs reported here, since the diagnoses in ≥ 3 lobes disagreed. These probabilities are applicable only to the study population which did not select dogs based on clinical evidence of liver disease. It is possible that different results would be obtained in dogs with a higher prevalence of liver disease.

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 4). These findings suggest that diffuse hepatic neoplasia commonly involves the majority of liver lobes, and is likely to be correctly diagnosed by biopsy of a single lobe. Caution must be used when extrapolating our results to the general population as most dogs had systemic round cell neoplasms that would likely be distributed more diffusely than primary hepatic tumors such as hepatocellular carcinoma. Additionally the sensitivity of a focal biopsy may be lower in cases of metastatic or focal neoplasia when a biopsy is not directed into a lesion identified visually or on ultrasound examination.[158]

Chronic hepatitis and fibrosis were the diagnosis least commonly present in all liver lobes. These data demonstrate the non-uniform distribution of histologic changes in both

of these conditions, and indicate that biopsy of multiple lobes may be necessary for their identification. Conflicting reports exist in humans about the distribution of fibrosis and inflammation between lobes. While some studies have reported substantial variation in fibrosis[160] and inflammation[161], others have shown minimal or no significant differences between lobes.[159,163,164] For example, a study comparing percutaneous liver biopsies obtained from both the left and right liver lobes in patients with hepatitis C virus showed no significant differences between grade and stage of disease between the two specimens.[162]

No predominant diagnosis could be determined in 10 cases (17.3 %) which had ≤3 lobes in agreement. Again this may lead to misdiagnosis if only one lobe is sampled and 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. Also because this study population included many dogs without clinically important liver disease, it is likely that the inconsistent distribution of findings would not reflect that of dogs with clinical signs from liver disease. One limitation of this study is that the 10 cases with ≤3 lobes in agreement could not be included in analysis of the data as no predominant disease was present.

Another potential limitation of this study is reliance on a single pathologist for interpretation of all of the liver samples. However use of a single pathologist likely resulted in more consistency in findings when compared with multiple observers.[177] Additionally, morphologic diagnoses were made based on standardized WSAVA

definitions which should also increase the consistency of diagnoses. Furthermore, the samples in this study were large samples from the center of each liver lobe, and were likely larger samples than what is collected by biopsy in clinical patients. This method was chosen to avoid artifacts that might be present in samples immediately adjacent to the liver capsule[107] and to ensure that tissue volume did not limit histopathologic interpretation. Histopathology from small needle biopsy samples in dogs has been shown to correlate poorly to larger surgical biopsy samples.[86] In this study relatively large liver samples were collected in order to ensure accurate histopathology. Therefore the proportion of discordant results between liver lobes may be higher in clinical patients than results from our necropsy population suggest. 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.

To the authors' knowledge, this is the only study to evaluate the variability in histopathology results between liver lobes in dogs. A previous study of hepatic lesions present in dogs at necropsy[178] reported a lower proportion of cases with chronic hepatitis, vacuolar change, and unremarkable samples as compared to our population. This discrepancy is likely due to the use of a single biopsy site (left lateral lobe) in the previous study. The higher of histopathologic abnormalities in our population likely reflects the proportion of dogs that have non-uniform changes throughout the liver and were therefore not accounted for in the previous study. However, when the present results from only the left lateral lobe are compared with results from the previous study, our population still had a greater proportion of chronic hepatitis and vacuolar change. This

would suggest that perhaps our population of dogs simply had a higher prevalence of these histopathology abnormalities.

During ultrasound guided percutaneous needle biopsy the left lateral lobe is most commonly sampled due to its relatively large size and distance from biliary structures.[30] 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, while total discordant results did not differ between lobes, the total proportion of discordant results was highest in the caudate lobe. It is possible that a larger sample size of livers examined would result in significantly more discordant results in the caudate lobe compared to the other lobes. This finding may be supported by a study of human patients with diffuse fatty infiltration of the liver in which 9% had disease which spared the caudate lobe only.[179]

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. Therefore biopsy of at least 2 liver lobes is recommended and result in a 98.6% likelihood of obtaining the predominant histologic abnormality in this study.

CHAPTER 4: CONCLUSIONS AND FURTHER RESEARCH

This study found no difference between the tested biopsy methods and all methods resulted in an unacceptably high proportion of discordant findings. The most likely explanation for the level of agreement is an inadequate amount of tissue collected by the biopsy method, as size was the primary variable between the biopsy and necropsy samples. However the punch biopsy samples had significantly more portal triads but not significantly more concordant samples than the other tested biopsy methods. This finding suggests that there may be a minimum biopsy size required for concordant results that was not acheved by any of the biopsy methods. These data also demonstrate that the likelihood of obtaining a representative sample is increased when multiple liver lobes are biopsied. Therefore, collection of multiple biopsies from different lobes may be more important than the method of biopsy used. The limitations of the tested biopsy methods might be overcome by obtaining multiple biopsies, a larger single sample, or perhaps biopsies from multiple lobes.

In the clinical patient it is reasonable to recommend the least invasive method of liver biopsy, as all tested methods had similar agreement. Additionally, all three methods had high specificities in all categories of disease, therefore if a diagnosis is obtained it is unlikely to be a false positive. If the histologic diagnosis is not supported by additional clinical data or response to treatment, a second biopsy where a larger sample is obtained during laparotomy may be necessary. Finally, biopsy of at least 2 liver lobes is

recommended as sampling 2 lobes resulted in a 98.6% likelihood of obtaining the predominant histologic abnormality in this study.

Further research should include collecting multiple samples from a single and/or multiple lobes to test for improved concordance. Additionally, this work should be expanded to a population of dogs with a clinically relevant liver disease, as the distribution of lesions throughout the lobe or lobes may be altered when clinically significant disease is present.

Finally, liver biopsy recommendations in humans vary based on the etiology of the liver disease. In dogs, liver disease, particularly diffuse liver disease, is less well-defined and frequently the etiology is unknown. Knowledge of the cause and ideal treatment of liver disease in the dog is likely necessary for ideal biopsy interpretation and collection recommendations.

FOOTNOTES

a. Miltex, inc.® 8mm Baker's biopsy punch

b. R. Wolf ® 5mm laparoscopic biopsy forceps

c. 14G VET-core® biopsy needle

d. SAS/STAT® software version 9.2. (Cary, NC, USA).

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APPENDIX A: Tables

Table 1. Agreement of histopathology diagnosis for each biopsy type as compared to the necropsy samples.

		Biopsy types		
		Needle	Cup	Punch
% Agreement		66%	60%	69%
Cohen's kappa coefficient		0.59	0.52	0.62
Sensitivity		60.30%	54.70%	66%
Specificity		83.30%	77.70%	77.70%

Table 2. Measures of performance for each biopsy type stratified by morphologic diagnosis in the necropsy samples

Diagnosis	Number	Needle Biopsy				Cup Biopsy				Punch Biopsy			
		Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
Normal	18	0.83	0.75	0.53	0.93	0.78	0.68	0.45	0.9	0.78	0.81	0.58	0.91
Vacuolar	18	0.72	0.96	0.86	0.91	0.61	1	1	0.88	0.83	0.96	0.88	0.94
Neoplasia	8	0.75	1	1	0.97	0.63	1	1	0.95	0.87	1	1	0.98
Fibrosis	6	0.16	0.98	0.5	0.92	0.5	0.97	0.6	0.95	0.5	0.98	0.75	0.95
Chronic hepatitis	5	0.6	0.98	0.75	0.97	0.4	0.98	0.67	0.96	0.4	1	1	0.96
Congestion	5	0.4	0.98	0.67	0.96	0.2	0.98	0.5	0.94	0.4	0.97	0.5	0.95
Cirrhosis	5	0.6	1	1	0.97	0.8	1	1	0.99	0.8	1	1	0.99

Only morphologic diagnoses with ≥5 cases are shown. PPV (positive predictive value); NPV (negative predictive value)

Table 3. Mean scores and standard deviations for histologic features of each sample type

	Biopsy	hepatocellular atrophy	hepatocellular hypertrophy	biliary hyperplasia	ceroid lipofuscin	hemosiderin	cholestasis (bile)	congestion	extramedullary hematopoiesis	vacuolar change	fibrosis	tissue inflammation	lobular collapse	necrosis	thrombosis	vascular abnormalities
Mean Score	Needle	0.13 SD=0.43	0.18 SD= 0.45 (P=0.017)	0.27 SD=0.67 (P=<0.001)	0.87 SD=0.95	0.71 SD=0.95 (P=0.0033)	0.37 SD=0.79	0.19 SD=0.50 (P=<0.001)	0.154 SD=0.41	0.88 SD=0.95	0.43 SD=0.80	0.32 SD=0.64	0.13 SD=0.06	0.28 SD=0.59	0	0.078 SD=0.32
	Cup	0.16 SD=0.49	0.34 SD=0.66	0.32 SD=0.73 (P=<0.001)	1.0 SD=0.92	0.75 SD=0.87 (P=0.02)	0.43 SD=0.82	0.25 SD=0.54 (P=0.0021)	0.11 SD=0.31	0.76 SD=0.87	0.60 SD=0.97	0.37 SD=0.68	0.15 SD=0.53	0.34 SD=0.62	0	0.15 SD=0.44
	Punch	0.19 SD=0.52	0.17 SD=0.45 (P=0.039)	0.45 SD=0.90	0.92 SD=0.75	0.79 SD=0.82 (P=0.024)	0.49 SD=0.87	0.37 SD=0.67	0.18 SD=0.052	0.91 SD=0.13	0.49 SD=0.95	0.39 SD=0.79	0.21 SD=0.60	0.33 SD=0.61	0.015 SD=0.12	0.18 SD=0.57
	Necropsy	0.19 SD=0.45	0.29 SD=0.66	0.58 SD=0.97	0.96 SD=0.76	0.95 SD=0.92	0.57 SD=0.90	0.48 SD=0.73	0.30 SD=0.57	1.01 SD=1.1	0.64 SD=0.12	0.41 SD=0.80	0.24 SD=0.55	0.41 SD=0.72	0.014 SD=0.12	0.14 SD=0.46
	Linera GEE P-value	0.66	**0.012**	**<0.001**	0.37	**0.015**	0.068	**<0.001**	0.052	0.11	0.058	0.4	0.56	0.29	0.56	0.55

Significant differences are shaded

Table 4. Proportion of diagnoses present in 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%
Vaculolar	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%
Necosis	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 Anomly	1		100%				

Table 5. Discordant results in each liver lobe

Diagnosis	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)	
Vaculolar	1 (Unremarkable)		2 (Unremarkable; Cirrhosis)				
Fibrosis		1 (Chronic hepatitis)	2 (Congestion; Unremarkable)		1 (Reactive hepatitis)	1 (Reactive hepatitis)	
Congestion	1 (Congestion)			1 (Unremarkable)			
Necosis					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 Anomly					1 (Congestion)	1 (Congestion)	
Total discordant results	6	2	5	2	5	9	P = 0.22

The left column shows the diagnosis in the discordant lobe. The predominant histopathologic abnormality in the remainder of the liver lobes is listed in parenthesis