THE C.L. DAVIS FOUNDATION AND THE AMERICAN COLLEGE OF VETERINARY PATHOLOGISTS PRESENT:

PATHOLOGY OF THE LIVER:
WHAT’S NEW AND WHAT’S STILL TRUE

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PATHOLOGY OF THE LIVER:
WHAT’S NEW AND WHAT’S STILL TRUE

8:00 AM to 5:00 PM  Saturday, November 10, 2007

8:00 – 8:15  Introduction

8:15 – 9:45  Introduction to liver disease; comments on WSAVA group
Hints on how to examine the liver biopsy slide
Special stains
Relationships between internist, radiologist, surgeon, pathologist

9:45 – 10:15  Break

10:15 – 11:45  Circulatory disorders
Congenital biliary disorders
Introduction to other biliary and parenchymal disorders

11:45 – 1:15  Lunch

1:15 – 1:30  Sponsor

1:30 – 3:00  Biliary Diseases
Parenchymal Diseases

3:00 – 3:00  Break

3:30 – 5:00  Neoplasia
Unknowns
Summary
PATHOLOGY OF THE LIVER: WHAT’S NEW AND WHAT’S STILL TRUE

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TEAMWORK

Before you even see the biopsy you can prepare in a way that will enhance the likelihood that the slide review will be rewarding for you and the clinician. Although not possible in all settings, it is very useful to establish a relationship with the clinicians (internists, radiologists) who submit liver biopsies. A dialogue with clinicians to encourage a full history and clinical findings is very helpful. For example, congenital vascular disorders tend to have a stereotypic histologic appearance and the final diagnosis can only be made with clinical information indicating the presence or absence of a shunt vessel.

It would be useful to encourage submitting clinicians to provide the following:

- Species, breed, age, gender
- Brief History
- Overview of clinical signs and findings
- Levels of biochemical alterations
  - Using specific numbers, not imprecise terms such as “elevated”.
- Why was the biopsy done?
- What did the liver look like?
  - An image from surgery or laparoscopy can be helpful
- Why was the biopsy done?

You may also provide suggestions on the method of biopsy. Diffuse conditions lend themselves to smaller biopsies. For example, hepatocellular steatosis would be easily diagnosed with a needle biopsy or aspiration. However, nodular lesions or many multifocal processes could easily be missed or the relative proportion of the liver affected can be under or over estimated. In these cases a wedge biopsy taken via laparotomy would be advantageous. Comparisons of needle biopsy with wedge biopsies from the same lobe revealed discordant diagnoses for about 50% of cases (Cole et al. 2002). Toxic liver injury may vary considerably within the affected liver with some areas of massive necrosis and local areas with scant centrilobular necrosis and a single needle biopsy can easily lead to the wrong conclusion regarding the overall status of the liver. In general, “more is better” and making this clear to the clinician can save frustration in selected cases.

Clinicians should be aware of appropriate guidelines for biopsy collection. Collecting biopsies at the wrong time or for the wrong reason can often lead to nondiagnostic biopsies. Some suggestions for appropriate collection are listed below.
• Abnormal serum enzymes and function tests -30 days or more
• Hepatomegaly of undetermined cause
• Hepatic involvement in systemic disease
• Staging of neoplastic disease
• Evaluation of response to therapy or progression of disease

When these guidelines are not followed a mismatch between clinician expectations and pathologic findings can result. The decision to biopsy an animal on the basis of a single observation of elevated transaminases is a good example. Take the following scenario. A dog ingests a hepatic toxin and liver injury develops in 24-48 hours. The animal is ill and taken to the clinician who learns that the liver-related enzymes are increased and schedules a biopsy the next day. Given the half life of ALT the dog may have significantly elevated enzymes three days after the injury, but the hepatic repair process can clear the necrotic hepatocytes and replicate to replace the lost cells yielding a relatively normal looking biopsy. The clinician points out that the ALT is 1000 IU/L, yet you say the liver is normal and suggests that not all Board certified pathologists are competent.

Proper handling of the biopsy at the time of collection is important. The biopsy should be immediately processed in the appropriate fashion (see below). Typically, the biopsy should be fixed at the time of collection, not warmed under the surgical lamps while the incision is closed. Drying and early degeneration adversely affect biopsy quality. Also, the excessive use of forceps to collect the biopsy can introduce significant crush artifact in small biopsies. It is also helpful to know that the clinician may be looking for before the biopsy is collected.

Samples may be better suited for diagnostics if they are not immediately placed into formalin. Some samples should be frozen for immunohistochemistry, depending on the antigen. Some, although not routine, biopsies may benefit from culture before they are fixed, if there is good evidence to suspect bacterial infection. Electron microscopy can be better performed if appropriate fixatives are used initially. Fat stains can not be done if the tissue has been processed and portions of samples may be held back in formalin for subsequent lipid evaluation. Molecular analyses are best performed on samples that have been snap frozen and held at -70˚ or placed in solutions designed to stabilize RNA (RNAlater).

Biopsies should be protected before transportation to the pathology laboratory. Needle biopsies should be transported in plastic cassettes or protected in surgical gauze as free floating biopsies can be fragmented in transit. Some plastic sponges used to protect biopsies have rough or pointed cut surfaces and can impale the specimens leaving a series of artifactual perforations in the liver and should, therefore, be avoided.

THE SLIDE

A review of the biopsy at the level of the unaided eye can be very useful. Initially, it is important to be sure the sample on the slide matches the description of the tissue trimmed in to be sure you have the right case. Also, since small samples may not settle evenly into the paraffin, it is useful to see if all the tissue
trimmed is present on the slide and that there is not a portion of the sample submerged in the paraffin and not yet cut. This is more common with fragmented needle cores and potentially useful tissue may not make it to the slide on the first effort. It is also useful to census all of the pieces of tissue on the slide to ensure that you have looked at all the tissue provided.

Clearly, there is no one correct search pattern for reviewing a liver biopsy, but a systematic approach is useful, as in the necropsy, and ensures that you have reviewed all relevant aspects of the submitted tissue. An approach that I use is to initially do the 1X naked eye review as described above and check for the uniformity (or lack thereof) of the sample and the color of the sample. Uniform red of the H&E of the liver suggests normal liver or smaller lesions. Pallor foreshadows vaculolated liver and areas of basophilia indicate the presence of infiltrating cells or possibly biliary proliferation. It gives you a starting point in the diagnostic decision tree. At low power a survey for the presence or absence of the lobular architecture is useful to assess uniformity of what ever process-parenchymal collapse, fibrosis, infiltration-may be occurring. I evaluated the central and sublobular veins next primarily because most attention generally goes to the portal tracts and the parenchyma and this region is often overlooked. Review of the portal tracts includes a census of all of the expected elements, portal vein, hepatic artery, bile ducts, lymphatics and nerves. Occasionally, portal vein profiles will be missing, but not detected if there is significant inflammation present.

Hepatocytes are evaluated for size (atrophy or hypertrophy), uniformity, vacuolization and zonal variation etc. Pigment deposition can be important. The presence of pigment granules in hepatocytes should generally be followed by application of special stains to assess the presence of copper, iron and lipofuscin or the proportions of each. Copper is a special challenge due to the inconsistent responses of the liver to different levels of copper and copper levels should be determined. (Note that if you suspected copper you would have saved a portion of the biopsy before it was fixed-see above). In my experience bile is rarely histologically evident in the cytoplasm of hepatocytes in dogs and probably cats, as well. If you suspect intrahepatic cholestasis in the absence of massive evidence of cholestasis in other areas of the liver, it would be advisable to confirm this with special stains. Similarly vacuolar contents can be identified in hepatocytes. In older dogs the decision is limited to lipid or glycogen, but in young animals, especially those with a history of poor growth rate, vacuolar contents may hold clues to the presence of storage disorders. Biopsies that have been collected and frozen, rather than fixed may be useful for biochemical analysis in these circumstances.

Kupffer cells can provide clues to the systemic disease as well as local disease processed. They should be evaluated for evidence of hypertrophy, pigment, infectious agents and erythrophagocytes among other issues.

**Special Stains/Diagnostic Techniques**

Finances may limit the decision to perform special stains in routine practice. When possible, a fuller overview of the status of the liver can be obtained with a modest battery of special stains. Stains for fibrosis can be informative, particularly if you are able to obtain serial biopsies to assess the progression of
fibrosis in chronic disease processes. There are several choices available. In our lab we prefer a routine panel of the following: Sirius red as it detects fine strands of collagen in early fibrotic processes, but Masson's Trichrome and other stains are also useful. Reticulin stains such as Gordon and Sweet are valuable for assessing lobular collapse. Stains for pigments; iron with Perl's Prussian Blue, copper with Rhodanine, lipofuscin with Schmorl's (and/or Ziehl-Neelson and PAS/diastase) and glycogen or other carbohydrates with PAS +/- diastase. Congo red for amyloid and stains for bilirubin are also useful. When infectious agents are suspected we include Acid fast stains including Fite’s stain, and various silver stains. Given the profusion of antibodies for infectious agents, diagnostic immunohistochemistry is progressively more useful, but forethought must be given to tissue collection as frozen sections maybe required or formalin fixation should be limited to 48 hours or so to prevent cross linking of epitopes. Molecular methods can be useful as well. For example, the distinction between lymphocytic cholangitis and lymphoma in older cats with marked lymphocytic infiltrates of the portal tracts can be a challenge. Samples can be assessed for monoclonality based on PCR results from T-cell receptor genes or immunoglobulin recombination sites improving diagnostic accuracy. Although these tests can be run of fixed and embedded samples, frozen tissue would be preferred.

Bibliography

Bunch, SE. Essentials of Small Animal Internal Med 1992


CONGENITAL VASCULAR DISORDERS OF THE LIVER
John M. Cullen VMD PhD DACVP

HISTOLOGIC PATTERN OF PORTAL VEIN HYPOPERFUSION

The liver responds to insufficient portal blood flow in a relatively stereotypic fashion regardless of the impediment to normal perfusion. As would be expected, diminished or absent portal flow is manifest by absent or diminished portal vein profiles in the portal tracts. In view of the normal balance of portal vein and hepatic arterial blood flow, it could be anticipated that there is an increase in arterial flow in livers that lack portal flow. This can be seen as an increase in arteriolar profiles in the portal tracts due, most likely, to an increase in arteriolar tortuosity and increase in artery branches and hypertrophy of existing arterioles, including the rete that normally surrounds bile ducts and the arteriole branches that extend into Rappaport zone 1 and 2 of the acinus. Periportal foci of sinusoidal dilation may also occur, presumably due to the local increased blood pressure that accompanies the arteriolar blood flow into the sinusoids. Bile duct proliferation may be evident, as well. Recent research has shown that bile duct epithelium responds to vascular endothelial growth factor (VEGF) and can synthesize it as well. In an environment where arterioles are proliferating or have proliferated and VEGF is likely to be present, the proliferation of bile ducts can be explained. Given the diminished inflow of nutrients and growth factors hepatocytic atrophy is also common. It seems likely that the distribution of what portal flow that is present is not distributed equally and the presence of abundant lipogranulomas probably represents the residuum of hepatocytes that have died due to insufficient perfusion, although other explanations are possible. Thus the final picture is one of lack of portal vein profiles, arteriolar proliferation, bile duct proliferation and hepatocellular atrophy with an excess of lipogranulomas in some instances.

Since the gut can be interpreted as a mere tube that conducts the nutrients that sustain the liver and the agents that intoxicate it, shunting of portal blood to the systemic circulation would be expected to have two major physiologic effects. The liver (and often the entire patient) would be small due to the lack of trophic influences-nutrients and growth factors-limiting hepatic function - and the patient would be intoxicated due the portal blood which is normal filtered by the hepatic sinusoids cleansing it of endotoxin and other byproducts of digestion, passing into the systemic circulation directly. The degree of clinical affliction likely varies with the proportion (shunt fraction) of portal blood diverted to the systemic circulation.
**Congenital Portosystemic Shunts**

Congenital portosystemic shunts are single (almost always) large caliber connections between the portal vein and any of a number of systemic veins. Shunts have been described in several species, but occur most commonly in the dog and cat. Affected animals are typically stunted and frequently develop signs of hepatic encephalopathy. A congenital shunt can be either intrahepatic or extrahepatic in location. A variety of different shunts have been described. Most often intrahepatic portosystemic shunts involve failure of closure of the ductus venosus at birth. The ductus venosus is a normal fetal vessel that conducts blood from the placenta to the caudal vena cava directly, since this blood contains the products of maternal metabolism and fetal hepatic metabolism is not required. Also, the small vascular bed of the developing liver is not likely to be able to handle the volume of blood flow. This anomaly occurs most often in large breed dogs. Extrahepatic congenital shunts, such as portal vein to caudal vena cava anastomoses and portal vein to azygous vein anastomoses, occur more often in small breeds of dogs.

The liver is small and may have a characteristic histologic appearance of lobular atrophy and reduplication of arterioles and small or absent portal veins within the portal tracts. The portal vein pressure is normal in congenital shunts and ascites does not occur because portal blood pressure exceeds the pressure in the vena cava (otherwise blood would not flow through the liver and out into the vena cava via the hepatic vein). If a congenital shunt exists any increase in pressure in the portal vein would drive blood flow into the systemic venous drainage preventing hypertension. Abnormal venous anastomoses are often difficult to identify at post mortem without benefit of antemortem imaging studies. Affected dogs frequently have abnormal plasma ammonia concentrations and, as a consequence, pass ammonium biurate crystals in their urine. Given the stereotypic response to inadequate portal vein perfusion, the histologic appearance of congenital portosystemic shunts and other vascular anomalies of the liver (discussed below) have considerable overlap. Clinical data, such as the presence or absence of shunt vessels and the determination of portal vein pressure, may be necessary to achieve a final diagnosis.

**Congenital Vascular Disorders Associated with Portal Hypertension**

*Intrahepatic arterio-venous fistulas*

Intrahepatic arterio-venous fistulas, either acquired or congenital, occur in the dog and cat. These shunts arise from a direct communication between a branch of the hepatic artery and branches of the portal vein. They may occur anywhere within the liver and a distended throbbing lesion can often be appreciated at surgery. Affected portions of the liver contain convoluted thick-walled arteries and distended aneurismal portal vein branches with abnormal, thickened walls. Shunting of blood may lead to portal hypertension or reversal of the direction of portal blood flow, subsequent development of acquired portocaval shunts and ascites. Clinical signs vary in intensity, most likely in relationship to the caliber of vessels that are affected, and are probably the result of the degree of portosystemic shunting of blood that results. In practice, these lesions are usually diagnosed prior to biopsy or surgical removal of the affected lobe or lobes and the clinicians usually know what they are dealing with before the pathologist.
Primary Portal Vein Hypoplasia (Microvascular Dysplasia, Noncirrhotic portal hypertension)

Considerable confusion surrounds this condition and there are a number of different diagnostic terms in the literature that have been used to describe this abnormality. In fact, there may be more than one pathogenesis leading to this general condition, but given the lack of understanding of this condition, a single name, primary portal vein hypoplasia, has been proposed by the WSAVA working group. Portal vein hypoplasia is a congenital vascular anomaly that occurs in dogs and occasionally in cats. It is characterized by abnormally small extrahepatic or intrahepatic portal veins, which result in diminished hepatic perfusion by the portal vein blood flow and the potential for portal hypertension. Affected animals have small livers and the typical histologic pattern of portal vein hypoperfusion, small or absent portal veins, proliferated hepatic arterioles, and hepatocyte atrophy. This disorder resembles portosystemic shunts histologically, but affected animals often have portal hypertension and resultant ascites. Portal fibrosis and biliary hyperplasia occur in about half of the cases. Because of the histologic similarities between portal vein hypoplasia and congenital portosystemic shunts, clinical data, such as imaging studies to determine the presence of a shunt vessel, is often required to make final diagnosis from biopsy material. In the absence of a diagnosed shunt and particularly in the presence of portal hypertension the most likely diagnosis is portal vein hypoplasia.

Combinations

It is possible that some dogs have both portal vein hypoplasia and portosystemic shunts. Cases with shunts that can not adjust to the increased portal vein flow then ensues following ligation or occlusion of the shunt vessels may not be able to adjust to the increased flow due to the combination of vascular anomalies.

Incidental Vascular Disorders

**Peliosis hepatitis**

Peliosis hepatitis is defined as a random distribution of dilated vascular spaces in the hepatic parenchyma. Grossly, these areas appear as variably sized dark blue foci within the liver that vary from pinpoint to several centimeters in size. It occurs in old cats and occasionally dogs, where it can be mistaken for a vascular tumor, such as hemangioma or hemangiosarcoma. Peliosis hepatitis (telangiectasis-see below) is particularly common in cattle and apparently is of no clinical significance. The lesions may arise from local obstruction of portal vein flow with subsequent hepatocyte atrophy and expansion of the sinusoids in areas where hepatocytes have been lost (termed phlebectatic type, also called telangiectasis) or from focal hepatocytic necrosis (parenchymal type). The utility of the distinction into two types and the differences between peliosis and telangiectasis are probably too small or too unclear to warrant further consideration.
CONGENITAL BILIARY CYSTIC DISEASE
John M. Cullen VMD PhD DACVP

Congenital biliary cystic diseases are a complex and often confusing collection of conditions. In the high altitude overview they can all be attributed to an abnormality of the development of the primordial biliary ductular system arising from the ductal plate. The descriptive terminology has, so far, been best worked out in human liver pathology.

As the liver develops the primitive hepatoblasts surround veins that drain the yolk sac, primitive gut and the placenta. Over time a row of hepatoblasts, termed the ductal plate, that are immediately adjacent to the myofibroblasts and connective tissue surrounding the developing portal veins begin to form a double row of cells that then develops into a series of tubules at the margin of the connective tissue. Some of these tubules persist and become the bile ducts and the remainder, normally, fail to develop further and disappear. Development of the hepatic artery branches in the portal tract precedes evolution of the formed bile duct in the portal tract and is a critical component of the entire process. The entire process is initiated near the hepatic hilus and proceeds peripherally to the margins of the liver. This wave of development, to foreshadow, helps to explain why various lesions occur at different portions of the biliary tree.

Congenital cystic disease is characterized by dilation of portions of the biliary tree and associated fibrosis. Similar lesions are frequently found in the renal tubules as well. In humans congenital cystic biliary disease is divided into three major forms:

1. Autosomal recessive polycystic kidney disease (ARPKD) or childhood type of polycystic kidney disease
2. Autosomal dominant polycystic kidney disease (ADPKD) or adult type polycystic kidney disease, and
3. Caroli’s disease with autosomal recessive inheritance.

ARPKD tends to be characterized by microscopic abnormalities of the bile ducts. Typically there is formation of fibrotic portal tracts and abnormal, often dilated irregular profiles of biliary epithelial lined ducts. In extensive cases the liver is altered due to extensive bridging fibrosis and the proliferated ducts and these cases are termed congenital hepatic fibrosis. Congenital hepatic fibrosis can occur in dogs and cats. Often, the portal vein profile is absent in this condition. I have previously misdiagnosed this lesion in cats as a congenital vascular disorder, rather than a primary ductal plate anomaly. Affected dogs and cats can develop portal hypertension.
In ADPKD the liver contains multiple unilocular or multilocular biliary cysts that range from a few millimeters to several centimeters in diameter containing clear fluid. So called von Meyenburg complexes, discrete fibrotic areas with small, frequently irregularly formed bile ducts are found in affected livers. Caroli’s disease is characterized by saccular dilations of the larger intralobular, lobar or common bile ducts. Affected ducts often contain inspissated mucus, bile and mineralized concretions. Caroli’s disease is thought to be caused by an early defect in the formation of the bile duct system, while ARPKD (childhood type) is thought to represent an intermediate type of anomaly with abnormally formed bile ducts throughout the liver. ADPKD with the unilocular cysts and von Meyenburg complexes are believed to represent an abnormality that develops at the later stages of development of the biliary tree. The large cysts are thought to develop over time by continued secretion from the lining biliary epithelial cells (recently demonstrated), creating prominent masses in older animals. Although the pattern of lesions and inheritance in domestic animals is not as clearly separated in domestic animals, the WSAVA has proposed the following classification for congenital lesions of the biliary tree:

1. Congenital dilation of the large and segmental bile ducts (similar to Caroli’s Disease)
2. Juvenile polycystic disease/congenital hepatic fibrosis
3. Adult polycystic disease (including von Meyenburg complexes)

The well known biliary and renal cystic disorder of Persian cats does not have a phenotype that allows consistent classification. Affected cats with renal cysts may develop biliary lesions typical of either juvenile polycystic disease or adult polycystic disease or a combination of the two.
Normal anatomy. Bile canaliculi (formed by hepatocyte membranes) carry bile from the hepatocytes to the bile ductules (cholangioles or canals of Hering) at the edge of the portal area. The ductules drain into the interlobular bile ducts in the smallest portal areas (lined by cuboidal cells). These bile ducts drain into the intrahepatic bile ducts (lined by columnar cells), which unite to form the main hepatic ducts that form the common bile duct at the junction with the cystic duct (from the gallbladder).

Non-congenital / non-inherited biliary diseases can be divided into the following categories: 1) Gallbladder lesions, 2) Cholestasis, and 3) Inflammation (cholangitis and cholangiohepatitis). These conditions often occur in the context of other liver lesions involving the biliary system and the parenchyma of the liver.

GALLBLADDER LESIONS

Cholelithiasis is not common in any domestic species, but is most often reported in ruminants. We see soft to hard choleliths most often in cats and less commonly in dogs and they may lead to obstruction of the cystic, common, or hepatic ducts. When analyzed, they most commonly contain bile salts and bilirubin or related compounds. Inspissated bile may also cause bile duct obstructions. Choleliths and inspissated bile may be seen with cholangitis and cholecystitis, but the nature of the association is unclear.

Cystic mucosal hyperplasia is relatively common in older dogs, and the mucosa is thickened and contains variably sized cysts lined by hyperplastic columnar epithelium. The cysts contain mucus. This lesion is usually of no clinical significance, but it may be associated with gallbladder mucoceles that can block the cystic or common bile duct and sometimes lead to gallbladder rupture.

Mucoceles refer to a syndrome in dogs in which the gallbladder is distended with firm tenacious mucus or mucoid bile. The mucosa usually has cystic mucosal hyperplasia. Mucoceles may be incidental findings at postmortem, but usually are seen clinically because they have extended into and blocked the cystic or common bile duct. They may lead to gallbladder rupture. They seem to be much more common recently.
**Gallbladder infarcts** have been recently reported in dogs and seem to be much more common now than they were 20 years ago. The animals may have severe bile peritonitis due to rupture of the infarcted gallbladder. Histologically, there is transmural coagulation necrosis of the wall of the gallbladder with minimal inflammation. Thrombi and atherosclerosis are seen in some cases and are likely the cause.

**Cholecystitis** can be acute or chronic and may be associated with cholangitis, cholangiohepatitis, choledoliths, and rarely rupture of the gallbladder. Acute cholecystitis is usually neutrophilic, with or without fibrin and there may be mucosal erosions or ulcers. It has been reported to be caused by viruses (Rift Valley fever and canine adenovirus) and bacteria (*Salmonella* spp. in cattle). We occasionally culture bacteria from acute cholecystitis cases in dogs and cats. Chronic cholecystitis is usually lymphocytic or lymphoplasmacytic.

**CHOLESTASIS**

**Cholestasis** is a decrease in the hepatic secretory mechanisms that leads to an accumulation of substances normally secreted in the bile (such as bilirubin and bile acids) in the blood. Cholestasis can be a primary event or secondary to other diseases. Cholestasis can be categorized as either:

- **Intrahepatic cholestasis** (due to decrease or blockage of bile flow in the canaliculi) occurs in association with a wide spectrum of conditions affecting hepatocytes (lipidosis, necrosis, hepatitis, cholangitis, etc.) or increased bile production associated with hemolysis.

  or

- **Extrahepatic cholestasis** (due to blockage of bile ducts) due to luminal obstruction by bile calculi (gall stones) or inspissated bile (often associated with gallbladder mucoceles in dogs), or extraluminal compression due to neoplasia or inflammation (particularly of the pancreas or duodenum).
  
  Histologically the most prominent feature of intrahepatic cholestasis is canalicular bile plugs. Other lesions associated with the primary liver disease process are often present and there may be hepatocellular swelling and Kupffer cells may contain bile. In addition to the lesions seen in intrahepatic cholestasis, in extrahepatic cholestasis there may be edema and neutrophils in portal areas and ductal bile plugs. In chronic cases there are concentric rings of fibroplasia and fibrosis around bile ducts, proliferation of bile ducts, and eventually bridging portal-portal fibrosis.
INFLAMMATION

Cholangitis

Acute and chronic neutrophilic cholangitis is common in cats, less common in dogs, and can be seen in many species. The pathogenesis is thought to involve ascending bacterial infections, but proof of either bacteria or an ascending infection is often difficult to obtain. In the acute lesions there are neutrophils either in the bile ducts, between the biliary epithelial cells, or in close association with the bile ducts. Neutrophils may also be present in the adjacent parenchyma (cholangiohepatitis), with or without necrosis, and abscesses may also be present.

In chronic cholangitis, lymphocytes and plasma cells are often mixed in with the neutrophils, and varying degrees of bile duct hyperplasia and fibrosis are seen. Portal-portal bridging fibrosis is present in severe cases. Chronic cholangitis has been reported in cats associated with liver fluke infestation.

Destructive cholangitis in dogs is rare and has been reported in association with some viral infections and as an idiosyncratic reaction to some drugs (trimethoprim sulfa).

Cholangiohepatitis

Cholangiohepatitis can be acute or chronic and I use the term when there is cholangitis with extension of inflammation, with or without necrosis, into the parenchyma of the liver.

Feline Inflammatory Liver Disease

A group of pathologists and internists at Penn are conducting 3 related studies of inflammatory liver disease in cats. I will present some of our preliminary findings at the Workshop. The three studies are:

1. How many inflammatory cells and what types are present in “normal” cats of various age groups. Is there an association with inflammation in other organs, particularly in those organs drained by the portal vein?

2. A retrospective study of inflammatory liver lesions in cats seen in surgical biopsies to characterize the lesions morphologically, attempt to identify causes, and correlate the lesions and causes with the clinical findings.

3. A retrospective study of severe inflammatory liver lesions in cats seen at necropsy to characterize the lesions morphologically, attempt to identify causes, and correlate the lesions and causes with the clinical findings.
Introduction to Parenchymal Diseases
Parenchymal disorders of the liver (in dogs and cats) can be grouped into seven categories: 1) reversible injury (cell swelling, glycogen accumulation, and lipidosis), 2) storage disorders, 3) amyloidosis, 4) miscellaneous disorders of hepatocytes, Kupffer and stellate cells, 5) necrosis, 6) inflammation (acute, chronic, and miscellaneous forms of hepatitis), and 7) cirrhosis. Lesions involving the hepatocytes are the hallmarks of most of these disorders; however, inflammation, necrosis, fibrosis, and bile duct proliferation are not restricted to parenchymal disorders and may be prominent in primary biliary or circulatory disorders.

REVERSIBLE INJURY

Hepatocellular swelling
Hepatocellular swelling (hydropic change, cloudy swelling) is the first manifestation of most forms of injury to cells and occurs whenever cells accumulate water due to their inability to maintain ionic and fluid homeostasis. Mild to moderate hepatocellular swelling can be a difficult morphologic change to detect with the light microscope. In severe hydropic change, there is marked swelling with very pale staining cytoplasm arranged in thin strands, or small vacuoles in the cytoplasm.

Glycogen accumulation (also known as vacuolar hepatopathy and steroid-induced hepatopathy)
This condition occurs in dogs and is most often associated with hyperadrenocorticism (although other steroid hormones, drugs such as D-penicillamine, and a variety of stresses can cause these changes). The characteristic features of the condition are swollen hepatocytes with clear cytoplasm due to glycogen accumulation and thin strands of eosinophilic cytoplasm without displacement of the nucleus from the center. The distribution and the extent of the lesion vary markedly and can be diffuse, zonal, or involve individual cells. Periodic acid Schiff (PAS) staining with or without diastase may help to identify glycogen accumulation in mild cases. Other hepatic changes associated with glycogen accumulation are marginated neutrophils in small blood vessels and occasional foci of extramedullary hematopoiesis.

Hepatic Lipidosis (Steatosis, Fatty Change)
Lipidosis is a non-specific reversible form of cellular injury and different mechanisms account for the triglyceride (triacylglycerol) accumulation in the liver. General textbooks in human pathology use steatosis and fatty change interchangeably, whereas veterinary pathology textbooks and laboratory animal and toxicologic pathology texts use lipidosis and fatty change interchangeably. Lipidosis is also used to refer to lipid storage diseases and specific clinico-pathologic syndromes (feline hepatic lipidosis). Pathologists will continue to use lipidosis, steatosis, and fatty change (fatty liver) to signify the same change in hepatocytes.
Hepatic lipidosis is the accumulation of vacuoles of lipids in the cytoplasm of hepatocytes. Formalin-fixed paraffin-embedded tissues have empty vacuoles due to the loss of fat in processing. Frozen sections of unfixed or fixed tissue can be stained for fat (oil red O, Sudan IV) and are helpful in recognizing microvesicular lipidosis and in differentiating fat from other substances that may accumulate in vacuoles. Lipidosis should be described by distribution (zonal or diffuse), severity, and size of the cytoplasmic vacuoles (microvesicular, macrovesicular, or mixed). Microvesicular lipidosis (vacuoles smaller than the size of the nucleus) is typical of diabetes mellitus in dogs in which lipid accumulation begins in the centrilobular area, and in juvenile hypoglycemia of small breed dogs in which the vacuoles are very small and may be diffuse or centrilobular. Mixed microvesicular and microvesicular lipidosis is common in the syndrome of feline hepatic lipidosis.

STORAGE DISORDERS

Storage disorders in hepatocytes and Kupffer cells due to inherited metabolic disorders can have many morphologic appearances. Common findings include clear vacuoles, vacuoles with granular or hyaline material, or cytoplasmic yellow brown material, all representing storage of substances due to inherited metabolic abnormalities (usually enzyme deficiencies). These changes are usually non-specific and should be evaluated using frozen sections, plastic-embedded sections, or electron microscopy. Definitive diagnosis depends on either the identification of the storage product, the enzyme deficiency, or the defective gene. Some storage disorders (copper and cholesterol esters) may lead to necrosis, inflammation, and cirrhosis.

AMYLOIDOSIS

Amyloid in the liver is usually secondary or reactive (serum amyloid-associated) amyloid and appears as eosinophilic material in the space of Disse and sometimes in the walls of vessels and in portal areas. Deposition may be diffuse, zonal, or multifocal and frequently there is atrophy of the adjacent hepatocytes and dilation of the sinusoids. Special stains (Congo red or Stokes) may be needed to identify or confirm the presence of amyloid. Hepatic amyloidosis is commonly associated with inflammatory conditions in other organ systems; however, in breeds with a predisposition to amyloid deposition (Chinese Shar-pei dogs and Abyssinian, Siamese, and other Oriental cats) inflammation in other organs may be slight or negligible. Spontaneous or biopsy-induced liver rupture with hemorrhage and hemoabdomen may occur in amyloid-infiltrated livers.

MISCELLANEOUS DISORDERS OF HEPATOCYTES, KUPFFER, AND STELLATE CELLS

Hepatocellular cytoplasmic alterations include: eosinophilic cytoplasmic bodies, cytoplasmic protein droplets, swollen hepatocytes due to increases in smooth endoplasmic reticulum (“ground glass” appearance), empyroplasia, lipofuscin and ceroid accumulation, and copper and iron accumulation. Hepatocellular nuclear alterations include: “brick” inclusions, cytoplasmic invaginations, glycogen inclusions, and intranuclear inclusions (viral and lead).
Hepatic stellate cells (lipocytes, Ito or fat storage cells) are, for unknown reasons, more numerous and have larger vacuoles in older cats (the change occurs less frequently in older dogs). Following stimulation by cytokines and growth factors, normal stellate cells are converted to myofibroblasts and contribute to fibrosis, along with other (portal) fibroblasts.

Necrosis of Kupffer cells may be seen in sepsis and toxemia. Kupffer cells engulf red cells (erythrophagocytosis) and accumulate iron (hemosiderosis) when there is increased red cell turnover. Apoptotic cells may be phagocytized leading to eosinophilic cytoplasmic inclusions. Ceroid/lipofuscin is a yellow-brown, PAS positive, lipid breakdown product that accumulates in Kupffer cells and macrophages as a result of increased hepatocyte turnover due to necrosis / apoptosis. Foci of ceroid-laden macrophages are common and have been associated with aging, previous hepatocyte death, portocaval shunts and abnormal liver perfusion. When they are composed of foamy macrophages with ceroid pigment they are termed lipogranulomas. Foci without foamy macrophages are termed pigment granulomas. These foci may also contain iron, lymphocytes and plasma cells.

CELL DEATH (NECROSIS/APOPTOSIS OF HEPATOCYTES)

Hepatocytes may be killed by various insults including hypoxia, toxins, microorganisms, immunological events and severe metabolic disturbances. Cell death has been considered to occur through apoptosis or necrosis; however, recent evidence suggests overlap between both processes as moderate exposure to some toxins causes apoptosis whereas greater exposure may result in necrosis. Apoptosis is an active process of programmed cell death that results in shrinkage of the cell without loss of integrity of the cell membrane, and subsequent fragmentation. Necrosis involves cytoplasmic swelling and loss of integrity of the cell membrane and may result in coagulative necrosis or liquefactive necrosis. Coagulative necrosis is the result of sudden and catastrophic denaturation of the cytosolic protein and appears as swollen hepatocytes with acidophilic cytoplasm, preservation of the basic outline of the coagulated cell, and pyknosis, karyorrhexis or karyolysis. Liquefactive necrosis is the result of osmotic swelling and disintegration of hepatocytes and appears as loss of hepatocytes with subsequent collapse of the residual reticulin network and/or replacement by erythrocytes and eventually the presence of ceroid-laden macrophages. Necrosis may be followed by proliferation of Kupffer cells and infiltration of phagocytic cells with subsequent resorption and lysis of the necrotic cells. Necrosis may be focal, multifocal, confluent, bridging, massive, or piecemeal.

Response of the Liver to Hepatocellular Injury

Following destruction of hepatic parenchyma, regeneration of parenchyma, fibrosis, and ductular proliferation (bile duct hyperplasia) may occur. When hepatocyte destruction is limited and the reticulin network remains intact, regeneration with almost complete restitution of the liver structure can occur. Severe parenchymal destruction with extensive loss of hepatocytes often is followed by ductular proliferation. With persistent parenchymal damage or extensive loss of hepatocytes and damage to the normal collagenous structure, fibrosis and postnecrotic scarring may occur which may result in regenerative parenchymal nodules. In areas of collapse and/or fibrosis, intrahepatic portovenous vascular shunts may form.
Controversy still exists about the nomenclature of hepatic necrosis and acute inflammation. We agreed that a morphologic diagnosis should emphasize the primary or most important process (necrosis vs. inflammation) with appropriate modifiers indicating chronicity, severity, distribution, presence of inflammatory cells, and evidence of organisms (inclusion bodies, bacteria, fungi, etc.). In some cases (ischemia, toxins, and certain viruses) zonal necrosis may be all that is seen in very acute conditions, but inflammation is likely to follow if the animal survives the initial insult. Similarly, in acute infections with some viruses and bacteria there is very little inflammation in the very acute phase, but traditionally these conditions have been referred to as hepatitis.

**Toxic liver injury**

Manifestations of liver toxicity, one or more of which may occur with each toxin, include: no morphologic abnormalities, hepatocellular swelling, lipidosis, necrosis (usually in a specific pattern), inflammation, and eventually fibrosis. The pattern of necrosis depends on many factors and the identity of the toxin cannot be determined based on morphologic grounds alone and should be verified by toxicological testing. Patterned necrosis of the liver can also be caused by vascular disease, hypoxia, and some viruses and must be distinguished from necrosis due to toxins.

Many hepatotoxins affecting dogs and cats are therapeutic agents that have idiopathic or idiosyncratic effects on the liver. Examples of acute toxicity include: centrilobular to panlobular necrosis in cats associated with benzodiazepines and in dogs associated with trimethoprim sulfonamide, and acute necrosis and inflammation in dogs associated with xylitol, carprofen and amiodarone. Chronic intoxication, sometimes leading to cirrhosis in dogs has been associated with primidone, phenytoin, phenobarbital, and CCNU (Lomustine).

**INFLAMMATION**

Hepatitis is inflammation of the liver parenchyma, whereas cholangitis is inflammation of the bile ducts. Inflammation is a complicated process involving the reaction to injury by blood vessels and the subsequent accumulation of fluid and leukocytes. Inflammation of the liver parenchyma can be a primary event or can follow injury to the hepatocytes. Injuries to the hepatic parenchyma may lead to reversible or irreversible injury. Reversible injury (cell swelling and lipidosis) rarely leads to inflammation; however, irreversible injury (cell death) is often followed by inflammation. Common types of cell death in the liver are apoptosis, coagulation necrosis, and liquefactive (lytic) necrosis. The outcome of a given hepatic insult depends on the nature, extent, and duration of the insult, and of course, survival of the host.

Hepatitis should be defined histologically by: severity, distribution, types of inflammatory cells present (neutrophils, eosinophils, macrophages, lymphocytes, plasma cells), presence or absence of necrosis and apoptosis, type of necrosis (liquefactive or coagulative), pattern of necrosis (multifocal random, zonal, massive), regenerative response of hepatocytes and bile ducts, presence or absence of fibrin or thrombi, evidence of causative agents (inclusion bodies, bacteria, fungi, copper, etc.), and presence or absence of fibrosis. The pattern of injury and the response to injury may provide clues as to the cause and/or pathogenesis. The chronicity of inflammatory lesions may be difficult to determine as the line of demarcation between acute and chronic inflammation may be blurred.
Acute hepatitis

Acute hepatitis is characterized morphologically by a combination of inflammation, hepatocellular apoptosis and necrosis, and, in some instances, regeneration. The proportion and detailed nature of these components vary widely according to the cause, the host response, and the passage of time. The lesions are usually sufficiently diffuse within the liver that a diagnosis of acute hepatitis can be made on small biopsy samples; however, although there may be histological clues suggesting a specific cause, it may be difficult to determine the cause by morphological means alone.

Specific causes of hepatitis in the dog include infectious canine hepatitis, canine herpesvirus, *Clostridium piliforme* (Tyzzer’s disease), leptospirosis, *Helicobacter canis*, and many other bacteria including *E. coli*, Streptococcus, Pasteurella, Salmonella, and Brucella, as well as *Toxoplasma gondii* (4).

Chronic hepatitis

Chronic hepatitis is characterized by hepatocellular apoptosis or necrosis, a variable mononuclear or mixed inflammatory infiltrate, regeneration, and fibrosis. The proportion and distribution of these components vary widely and it is necessary to include in the diagnosis the activity and stage of the disease as well as the possible cause. The activity of the disease is determined by the amount of inflammation and extent of hepatocellular apoptosis and necrosis. The stage of the disease, and the prognosis, may be determined by the extent and pattern of fibrosis and the possible presence of architectural distortion. Fibrosis may be porto-portal, porto-central and centro-central bridging or it may dissect the lobule. It may occur associated with interface hepatitis (inflammation at the limiting plate), following collapse and condensation of the reticulin network, or by direct activation of hepatic stellate cells with perisinusoidal deposition of collagen. Regeneration and regenerative nodules of hepatic parenchyma are often seen as well as proliferation of ductular structures at the periphery of the parenchyma and within fibrous septa. Histochemical stains for connective tissue may be helpful in detecting the amount and pattern of fibrosis, particularly in early and mild disease.

The cause of most spontaneous cases of canine chronic hepatitis is undetermined although some cases have been associated with leptospirosis, *Bartonella* spp., and experimental and spontaneous infectious canine hepatitis virus infection. Chronic hepatitis has also been reported in dogs treated with anticonvulsant drugs such as primidone, phenytoin, and phenobarbital, and in aflatoxicosis.

Copper-Associated Chronic Hepatitis

In the Bedlington terrier a genetic mutation in copper transport proteins causes accumulation of copper in hepatocytes resulting in inflammation or necrosis. Copper accumulation leading to inflammation and necrosis appears to be familial in the West Highland white terrier, Skye terrier, and Dalmatian and has recently been reported in other breeds (Labrador retriever). In these animals copper accumulates in hepatocytes, often starting in the centrilobular regions, and with progressive accumulation results in hepatocyte necrosis, inflammation with copper-laden macrophages, and eventual chronic hepatitis and cirrhosis.
Healthy dogs with normal livers may have copper levels up to 500 micrograms per gram dry weight. Hepatic copper levels in breeds with primary copper storage disease vary between individual animals and between breeds. Bedlington terriers, West Highland white terriers and Dalmatians with copper associated liver damage reportedly have liver copper concentrations greater than 2000 micrograms per gram of dry weight liver. Affected Skye terriers have concentrations ranging from 800-2,200 micrograms per gram. Other breeds (Doberman pinschers, Labrador retrievers, and American and English cocker spaniels) have been reported to have elevated copper concentrations in association with chronic hepatitis but it remains to be determined whether this copper accumulation is primary or secondary to chronic inflammation, fibrosis, and cholestasis.

Copper-induced chronic hepatitis and cirrhosis was recently observed in cats and was characterized by severe copper deposition in hepatocytes and macrophages in the fibrotic areas and slight to moderate copper deposition in the regenerative nodules.

**Non-specific reactive hepatitis**

Non-specific reactive hepatitis is a morphological entity that is often widespread within the liver, and represents a non-specific response to a variety of extrahepatic disease processes, especially febrile illnesses and inflammation somewhere in the splanchnic bed. It may also be the residual lesion of previous inflammatory intrahepatic disease. The lesion is characterized by an inflammatory infiltrate in portal areas and in the parenchyma without evident hepatocellular necrosis. In acute extrahepatic diseases there is a slight to moderate infiltrate of mainly neutrophils in the stroma of the portal areas that varies in intensity between portal areas. (Some portal areas may be normal.) There is slight to marked leukocytosis, Kupffer cell proliferation in the sinusoids, and some neutrophils in the stroma around the hepatic veins. In chronic extrahepatic diseases or in cases of residual intrahepatic disease, the inflammation usually is mainly mononuclear with plasma cells and lymphocytes (and pigmented macrophages) in the portal areas and around the hepatic veins as well as some plasma cells and lymphocytes, with or without single or small aggregates of pigment laden macrophages within the parenchyma.

**Hepatic abscesses and granulomas**

Hepatic abscesses usually are the result of bacterial infections evoking intense accumulation and subsequent lysis of neutrophils. Bacteria can reach the liver via different routes including the portal vein or umbilical vein, ascending infection of the biliary system, and by direct contact and penetration of the liver capsule. Hepatic abscesses in dogs and cats are particularly seen in newborn animals due to umbilical infection by Gram-positive and Gram-negative bacteria. In adult animals hepatic abscesses may be the result of infections with *Yersinia spp*, *Nocardia asteroides* and *Actinomyces spp*. Hepatic abscesses may occur in association with central necrosis in hepatocellular neoplasms.

Hepatic granulomas may occur in a wide variety of diseases, some of which are primary in the liver, but most are part of a generalized disease process. They consist of aggregates of epithelioid macrophages and/or multinucleated giant cells with or without lymphocytes and plasma cells.
Infectious causes for hepatic granulomas in dogs include mycobacterial infections
(*M. avium intracellulare, M. tuberculosis*), Bartonella spp., systemic mycoses
(*Blastomyces dermatitidis, Cryptococcus neoformans, Histoplasma capsulatum,
*Coccidioides immitis*) and opportunistic fungal infections, and migrating nematode larvae
(visceral larva migrans). A diffuse granulomatous inflammation of the liver is observed
in *Leishmania spp* infection in dogs. Granulomas may also be incited by relatively inert
foreign material (e.g. crystalline material, sutures, plant material). It is also seen in
neonates at the periphery of the liver lobes due to subcapsular hepatocellular necrosis
(probably associated with ischemia) with subsequent mineralization and granulomatous
inflammation.

**Eosinophilic hepatitis**

Eosinophils may be present as part of a mixed inflammatory infiltrate in portal and
perivenous areas and less frequently within the sinusoids. They can be regarded as a non-
specific reactive hepatitis particularly associated with allergic conditions and
hypereosinophilic syndromes. Marked eosinophilic inflammation in the liver is a rare
condition in dogs and may be associated with parasitic infections (migrating nematode
larvae) usually at and near the site of the parasitic lesion.

**Cirrhosis**

There are many and somewhat conflicting definitions of cirrhosis and some would
instead prefer the term “end-stage liver”. We recommend the following definition:
diffuse fibrosis with alteration in hepatic lobular architecture (usually with the formation
of nodules) and portal-central vascular anastomoses. Cirrhosis is the end-stage of chronic
hepatitis and is a diffuse process characterized by fibrosis and conversion of normal liver
architecture into abnormally structured parenchymal nodules. In cirrhosis two
morphological categories can be distinguished i.e. micronodular cirrhosis with nodules
less than 3 mm and regular in size and macronodular cirrhosis with nodules greater than
3 mm and irregular in size. Micronodular cirrhosis develops from regular and diffuse
alteration and fibrosis while macronodular cirrhosis develops from irregularly distributed
larger areas of necrosis with secondary collapse and scarring. In dogs with cirrhosis,
proliferation of ductular structures is common, and varying degrees of inflammation, bile
stasis, lipidosis, and glycogen accumulation are also present. Ascites and multiple
acquired portosystemic shunts are common. Cirrhosis is much less common in cats. Cats
with diffuse hepatic fibrosis and disruption of the normal lobular pattern rarely have
nodules of regeneration. These cats may also have inflammation and proliferation of
ductular structures.

Hepatocutaneous syndrome is a syndrome in the dog in which there is skin disease
(superficial necrolytic dermatitis) in association with a form of cirrhosis. The liver in this
syndrome is divided into nodules by fibrous septa containing proliferations of ductular
structures and macrophages. The nodules are bordered by enlarged hepatocytes
containing clear vacuoles.
Lobular Dissecting Hepatitis

Lobular dissecting hepatitis is a form of cirrhosis with a rapid clinical course seen in young or young adult dogs as isolated cases or in groups of dogs from the same litter or kennel. The liver usually has a normal size with a smooth capsular surface or some small nodules of regeneration. Microscopically, bands of fibroblasts and thin strands of extracellular matrix are seen between individual and small groups of hepatocytes that cause dissection of the original lobular architecture. Connective tissue stains (especially for reticulin) are helpful in demonstrating the pattern of connective tissue alterations. Inflammation and hepatocellular apoptosis / necrosis are slight to moderate. The cause is not known.
Selected Recent References:

Books


Biliary


Parenchymal


Appendix: Guidelines for liver biopsies in dogs and cats (proposed for the Veterinary Hospital of the University of Pennsylvania)

The following guidelines have been formulated to answer common questions about liver biopsies and to facilitate communications between clinicians and diagnosticians. The guidelines are organized as follows:

1. Reasons for performing a liver biopsy
2. When and in what patients are liver biopsies recommended?
3. Appropriate samples and labeling and handling of samples for histopathology / cytology
4. Guidelines for cytological aspirate or imprint biopsies
5. Guidelines for ultrasound-guided liver biopsies
6. Guidelines for surgical liver biopsies
7. Appropriate samples and labeling and handling of samples for microbiology and toxicology (including metal analysis)
8. What you should expect and not expect from the pathologist and clinical pathologist

1. Reasons for performing a liver biopsy

   Academic interest
   - Diagnosis
   - Prospective studies to determine prognosis
   - Investigate liver development
   - Explanation for abnormal clinical signs, clinical chemistry findings, imaging results

   Patient interest
   - Diagnosis
   - Cause
   - Prognosis
   - Treatment

   Owner interest
   - Concern over unexplained results (abnormal clinical signs, clinical chemistry findings, imaging results)
   - Genetic / Congenital diseases
2. When and in what patients are liver biopsies recommended?

Liver biopsies are indicated in the following patients:

Those with clinical chemical abnormalities:

- Animals with persistent elevation of liver enzymes (ALT, AST, ALP, GGT) in the absence of another explanation (e.g. hyperthyroidism, hemolysis, muscle disease, drug induction, hyperadrenocorticism).

- Animals with persistently elevated post-prandial and/or fasting bile acids when portal-systemic vascular anomalies have been ruled out.

Those with radiologic abnormalities:

- Animals with unexplained hepatomegaly or microhepatica

- Animals with solitary hepatic masses

- Animals with presence of multiple liver nodules (nodular hyperplasia?)

- Animals with diffuse ultrasonographic abnormalities such as hypo- or hypechogenicity or a "mottled" appearance. Note: fine needle aspiration cytology may be warranted prior to biopsy when diffuse disease is present

Those undergoing exploratory surgery:

- Animals with gross liver abnormalities of any kind noted at surgery.

- Animals with primary neoplasia at another site in the abdomen, to rule out metastatic disease.

- Animals with hyperbilirubinemia with evidence of intrahepatic or extrahepatic cholestasis

- Animals with gall bladder mucocoeles

- Animals requiring surgery for portal vascular anomalies (i.e. portosystemic shunts)

Liver biopsies are contraindicated in the following patients:

- Those that are poor anesthetic candidates.
- Those with significant coagulation abnormalities (US-guided)
3. Appropriate samples and labeling and handling of samples for histopathology / cytology

All veterinarians involved in obtaining and evaluating liver biopsies should be provided with the signalment and a brief summary of the history including medication history, physical examination findings, preliminary test results and the reason(s) for performing a biopsy.

In general, the pathologist (either biopsy or cytology) would prefer to have:

- samples from different lobes if the liver is diffusely and uniformly affected
- samples of “normal” and abnormal areas if it is not diffuse
- all samples labeled (as site in the liver and normal vs. abnormal (unless the liver alterations are thought to be diffuse and uniform)
- as large a sample as is reasonable and intact samples rather than fragmented ones (biopsy)

Small samples and trucut and needle biopsy samples are easily fragmented in transport, especially if the liver is diseased, so these biopsy samples should be submitted either in small vials or mesh cassettes, without using plastic sponges, which tend to crush the samples.

4. Guidelines for cytological aspirate or imprint biopsies

As with needle biopsies, multiple samples from different lobes representing both abnormal areas and adjacent normal tissue, labeled accordingly, are ideally obtained. Generally, 3-5 smear preps per site aspirated is adequate.

Impression smears or roll preps can be prepared from biopsy material when tissue fragments are of sufficient size to permit manipulation without damage to the specimen. The specimen to be imprinted can be skewered on a needle to avoid crush artifact associated with handling by forceps. Once biopsy specimens have been placed in formalin, they are rendered unacceptable for cytological smear preparation and toxicology testing.

Slides should be air-dried and submitted unstained. Previously stained (Diff-Quik) smears are acceptable as an inferior alternative; however diagnostic insight is frequently diminished.

Caution should be taken whenever possible to minimize artifact from peripheral blood contamination, ultrasound lubricant gel contamination, and cell disruption. Formalin exposure (liquid and/or fumes) will dramatically alter cellular staining, thus cytological smears should be prepared and shipped in a formalin-free environment, separate from histological specimens.
5. Guidelines for ultrasound-guided liver biopsies

Multiple samples from different lobes can usually be obtained in animals over 10 Kg. In smaller patients or patients with reduced liver size, sampling is usually limited to one window. In these patients, the biopsy needle can be inserted in different directions from the same window to sample different areas of the same lobe.

Samples can be taken of abnormal and adjacent normal areas and labeled accordingly.

Samples will be labeled with the biopsy site and as lesion or normal liver tissue; if there is a focal mass, an ultrasound image can be submitted with the samples.

Size of the sample has to be adjusted to patient size, body condition and liver size.

Most liver biopsies are performed using a 14 gauge biopsy needle; however, the radiologist may choose a smaller needle (16 gauge) depending on patient size.

The biopsy sample should be flushed off the biopsy needle with saline into a vial containing an adequate volume of formalin.

Fragmented samples may be submitted for culture, or frozen for PCR or toxicology as needed; intact samples are submitted for histopathology.

6. Guidelines for surgical liver biopsies

In livers that are grossly uniform or diffusely involved in a disease:

At least two samples from different lobes of the liver should be obtained. Large wedges of liver should be obtained incorporating a substantial amount of hepatic tissue that is not at the periphery of a lobe. Individual samples may then be divided on the surgery table if necessary to submit for culture, frozen sections, PCR, or routine histopathology processing.

Samples should be snap frozen in liquid nitrogen if liver metabolic abnormalities are suspected from the medical investigation or preoperative metabolic screenings.

Samples are then labeled appropriately with the relevant patient information and the location (lobe) from which the sample was taken.

In livers with a focal lesion(s):

In general the affected lesion is submitted as part of a partial or completely resected lobe. The location (lobe) of the sample is labeled on the submission.

In animals with vascular abnormalities (portosystemic shunts):

The variability of histopathologic findings from different lobes in animals with portosystemic shunts remains to be elucidated, as does the prognostic significance of the findings. At least one wedge biopsy of the liver should be obtained until these issues are clarified.

Other considerations:

In feline liver biopsies, the surgeon should routinely also submit one or more small intestine biopsy samples because of the association between cholangitis and feline inflammatory small bowel disease.
7. Appropriate samples and labeling and handling of samples for microbiology and toxicology (including metal analysis)

**Bacteriology/Toxicology**

Abscesses or suppurative lesions (hepatitis/cholangitis/cholangiohepatitis)

Aerobic and anaerobic culture of liver lesion or bile (if cholangitis). Bile may be the more rewarding specimen and can be stored at −70 C prior to analysis.

Other inflammatory lesions

Frozen sample for toxicology/metal analysis (or PCR)

**Cryostorage of biopsy tissue**

For *long term storage* tissue should be maintained at temperatures below -130 ºC in order to preserve the material unaltered. This is achieved by storing tissue samples in specifically designed racks just above the liquid phase (-196 ºC) in an appropriate liquid nitrogen storage unit. Liquid nitrogen is a dangerous medium to work in as the 700X expansion on heating of the material in an enclosed space can lead to explosions.

It is a fact that no mechanical closure can be guaranteed in the liquid phase of nitrogen. This is why CryoFlex™ was introduced. When it is deemed necessary to store the material in the liquid phase, we advise the use of Cryo Tubes™ with an internal thread, and the correct application of CryoFlex™ to all tubes. This method is also recommended for SNAP FREEZING tissue samples for molecular diagnostics.

Remember to use safety equipment when handling liquid nitrogen. It is a matter of good laboratory practice to wear gauntlets and eye protection when handling this dangerous material.

**SNAP FREEZING PROTOCOL**

1. The tissue is placed into a sterile Nunc CryoTube™ labeled with the specimen number/case number. A barcode system is available from NUNC.
2. Cryo Flex™ is applied and tissue is SNAP frozen in liquid N2.
3. The tubes can then be transferred to the −70°C freezer for short term storage or stored in aluminum racks in the liquid N2.

**PCR**

Amplification of fragments >300bp from biopsy samples is not recommended (Bonin et al, J Clin Path: Mol Pathol. 2003:56, 184-86) therefore assays should be designed with this in mind.

Commercial DNA extraction kits are available and generally no more than 25 mg of tissue is required.

Biopsy samples should be fixed in a buffered formaldehyde solution (at a physiological pH) for no more than one week prior to DNA extraction.
Assays for specific organisms include:

- *Bartonella* - available at VHUP
- *Mycobacterium* - available at VHUP
- *Leptospira interrogans* - available at VHUP

**Toxicology** *(contact the Toxicology Laboratory for specific guidelines for individual tests)*

Findings that may be consistent with a toxic insult to the liver include:

- Hepatic lipidosis
- Necrosis
- Nodular regeneration
- Fibrosis
- Atrophy
- Changes in pigmentation

100 mg wet weight of liver = 1 good Trucut. This should be sufficient for most heavy metal analyses.

Liver can also be screened for insecticides, some plant toxins, some drugs, vitamins A and E, and other substances, though a minimum of one gram of tissue is required. Anticoagulant rodenticides can also be detected in liver, though it is unlikely that liver biopsies would be performed on these patients.

For acute intoxications the best sample may be whole blood or serum rather than liver.

**8. What you should expect and not expect from the pathologist and clinical pathologist**

You should expect:

- Timely results
- Consults (telephone, e-mail, etc.)

Pathologist

Morphologic diagnosis (severity, time, distribution, process)

A morphologic diagnosis should emphasize the primary or most important process (necrosis, inflammation, neoplasia, etc.) with appropriate modifiers indicating chronicity, severity, distribution, presence of inflammatory cells and fibrosis, and evidence of organisms (viral inclusion bodies, bacteria, fungi, etc).

A brief description that describes the sample and covers the same areas as the morphologic diagnosis
Comment on possible causes or associations

Suggestions for further diagnostic efforts (serology, additional history, culture, PCR, toxicology, etc.)

You should not expect:

Cause in most cases (particularly in chronic disease)
Prognosis in most cases
Treatment options

Clinical Pathologist:

Diagnostic Interpretation (Finding, severity, degree of confidence)

A diagnostic interpretation will include a listing of findings deviating from “normal” (inflammation, neoplasia, cellular vacuolation, infectious organisms, etc.) with appropriate modifiers indicating magnitude of change (few, many, mild, moderate, marked, etc.) and degree of confidence that the findings are representative (possible, probable, certain). When multiple alterations are present, these findings are ordered by degree of certainty and clinical relevance.

A brief description that elaborates on sample features, covering the same areas as the diagnostic interpretation.

Comments on potential causes or associations, and suggestions for further diagnostic efforts (immuno/cytochemical special staining, bone marrow aspiration, histopathological biopsy, culture, serology, PCR, etc.)

You should not expect:

Cytological diagnostic interpretations related to:
- Alterations in tissue architecture such as fibrosis, atrophy, well-differentiated neoplasia, or vascular anomalies.

- Size or distribution of lesions (i.e. periportal, centrilobular, multifocal, massive).
HEPATIC NEOPLASIA AND HYPERPLASIA
John M. Cullen VMD PhD DACVP

There are many cell types within the liver; however, the hepatocytes and biliary epithelium are the source of the majority of hepatic neoplasia. Tumors of the hepatobiliary system can arise from epithelial elements; hepatocytes, biliary epithelium of bile ducts or the gallbladder, and mesenchymal elements, such as connective tissue and blood vessels. The liver may be the most common site of metastasis and by far, the majority of neoplasms within the liver are metastases. The reader is recommended to review the WHO fascicle on Histological Classification of Tumors of the Alimentary System of Domestic Animals and the WSAVA Standards for Clinical and Histological Diagnosis of Canine and Feline Liver Diseases for more detailed descriptions and discussion.

Nodular hyperplasia

Nodular hyperplasia of hepatocytes is a very common proliferative lesion in dogs more than 8 years old and nearly all dogs are affected by 14 years of age. They are of no clinical significance, but should be distinguished from metastatic masses. Swine and cats have a low incidence of nodular hyperplasia and it is rare in other species.

Nodular hyperplasia of the liver is characterized by multiple distinct masses that are randomly distributed throughout the liver. The affected liver is usually normal otherwise. The nodules are spherical to oval and the cut surface is well delineated from the adjacent normal parenchyma. The lesion can be distinctly paler or darker than adjacent liver parenchyma depending on the amount of hepatocellular vacuolization of affected hepatocytes and the degree of congestion. Occasionally the lesions are difficult to detect due to their close resemblance to normal parenchyma. The nodules range in size from 2 mm to 3 cm in diameter. They are difficult to distinguish from hepatocellular adenomas by gross pathologic morphology alone.

Histologically, nodular hyperplasia is characterized by an expansile nodule of hepatocytes that retains normal lobular architecture. Portal tracts and central veins may be more separated than normal, but lobular architecture can be discerned. Although they often compress adjacent normal tissue so that there is a condensed zone of reticulin, the nodules are never encapsulated and there is never an increase in amount of fibrous tissue within the nodule. Hepatocytes are arranged into plates one to two cells wide. Within the nodule, vacuolization, when present, can be diffuse or focal. Vacuolated hepatocytes contain lipid or glycogen, alone or in combination. Generally hepatocytes in nodules of hyperplasia are enlarged because of increased cytoplasm or because of extensive vacuolization. Nucleoli are occasionally enlarged, but nuclei are usually normal, but Mitotic figures are uncommon, but may be more frequent than those found in normal liver.
Regenerative Nodules

Nodules of regenerative hyperplasia are distinct from nodular hyperplasia. Regenerative nodules occur in some species, most often dogs, as the result of chronic injury. Often the insult is unknown, but the response of some dogs to anticonvulsant drugs such as phenobarbital or phenytoin serves as an example. Regenerative nodules are unlikely to be confused with nodular hyperplasia since regenerative nodules arise in response to liver damage and significant fibrosis and the incidence is not related to age. Since these lesions are presumed to result from the outgrowth of surviving hepatocytes there is usually only a single portal tract within the regenerative nodules. Regenerative nodules can be difficult to distinguish from hepatocellular adenomas on the basis of histology alone, although there are a few distinguishing features. Regenerative nodules are composed of hepatic plates that are no more than two cells thick and adenomas may have thicker hepatic plates. Hepatocytes in nodules of regenerative hyperplasia may be focally or diffusely vacuolated with glycogen or lipid within the vacuoles. Hepatocellular adenomas are more likely to be solitary lesions and usually do not arise in a background of hepatic injury and fibrosis.

Hepatocellular adenoma

Hepatocellular adenomas are benign neoplasms of hepatocytes. They have most likely be underdiagnosed for many years by veterinary pathologists, as older terminology in dogs tended to include only nodular hyperplasia and regenerative nodules and hepatocellular carcinomas. The neoplasms usually are single, unencapsulated, variably sized, red or brown masses that compress adjacent parenchyma. They are typically spherical, but may be pedunculated. They are composed of well-differentiated hepatocytes, which form uniform plates that may be two to three cells thick. Hepatic plates in adenomas tend to abut normal adjacent hepatocytes at right angles. Diagnostic criteria to distinguish hepatocellular adenomas and hepatocellular nodular hyperplasia can be somewhat subjective because both arise in livers with no background abnormality, unlike regenerative nodules that arise in damaged livers. Histologically, adenomas are characterized by only one or very few portal tracts, whereas hyperplastic nodules retain normal lobular architecture elements, although the portal tracts are more separated than normal. In other cases, it may be difficult to distinguish hepatocellular adenomas from well-differentiated hepatocellular carcinomas and with time my perspective is that primary hepatocellular neoplasms tend to be called malignant more often than needed. Most of us have a very small list of cases with clear cut metastasis from hepatocellular neoplasms and rely on histologic criteria, rather than biologic behavior to diagnose malignancy.

Hepatocellular carcinoma

Hepatocellular carcinomas are malignant neoplasms of hepatocytes. They are uncommon in all domestic species, but may occur more frequently in ruminants, particularly sheep. These neoplasms are often solitary, frequently involve an entire lobe, and are well demarcated. They typically consist of friable, gray-white, or yellow-brown tissue, which is subdivided into lobules by multiple
fibrous bands. Malignant hepatocytes characteristically form irregular trabeculae three or more cells thick and vascular spaces are present between the trabeculae. Crude acini forming a pseudoglandular pattern of neoplastic cells are sometimes present. Within an individual tumor, trabecular, pseudoglandular, and solid patterns may be found. Cells present in the neoplasm range from well-differentiated hepatocytes to atypical or bizarre forms. In the absence of metastasis, which is obviously indicative of malignancy, distinction of well-differentiated carcinoma from adenoma can be difficult, although invasion by malignant hepatocytes at the margin of the adjacent compressed normal hepatocytes and hepatocellular atypia are useful indicators of malignancy. Metastasis to a variety of sites may occur, particularly to lymph nodes within the cranial abdomen, lungs, and seeding into the tissue of the peritoneal cavity. Some hepatocellular carcinomas spread extensively within the liver (intrahepatic metastasis).

**Cholangiocellular (biliary) adenoma**

Adenomas of the biliary ducts are uncommon in most species, but may be the most common primary hepatic neoplasm in cats, depending on the preferred diagnostic terminology, discussed below. They are usually small and asymptomatic, discovered as an incidental finding at necropsy, so they are found in old companion animals and in younger animals slaughtered for food. Biliary adenomas are nonencapsulated, irregular, pale white to pale gray, multilocular masses characterized by the formation of round acini, bile ducts or expanded spaces lined with well-differentiated biliary epithelial cells. The single layer of lining cells may be cuboidal, oval or flattened. The cytoplasm is usually pale eosinophilic and occasionally granular. Nuclei tend to be basally oriented with prominent nucleoli. The lumen of the acini or ducts often contains a pale eosinophilic to mucinous material. Mitoses are uncommon. The neoplastic epithelial cells are mounted on irregularly thick fibrous stroma. They are usually solitary. They grow by expansion sand therefore have distinct borders.

Cystic biliary lesions in dogs and cats can arise from inherited or congenital abnormalities of the embryonic primordium of the bile ducts, the hepatic ductal plate. These lesions may occur in conjunction with cystic changes in the kidney. Since there is little histologic difference between these lesions and descriptions for biliary adenomas, consideration should be given to the possibility of developmental (hamartoma), rather than a neoplastic process as the origin of these lesions (and biliary cystadenomas discussed below). When there are multiple cysts and islands of entrapped normal hepatocytes are found in the tumor stroma a developmental anomaly of the biliary tree should be considered as a differential diagnosis. Recent evidence suggests that the lining biliary epithelia of cysts maintain a secretory activity that produces a gradual distention of the early small ductal plate lesions so that over a number of years large fluid filled cysts appear. This may explain why cystic lesions are rarely seen in young animals unless careful microscopic examination is performed and why these cystic lesions are seen only later in life.
Cholangiocellular (biliary) carcinoma

Cholangiocellular carcinomas are malignant neoplasms of biliary epithelium, which usually arise from the intrahepatic ducts, but extrahepatic bile ducts can be affected. These neoplasms occur in all species. Tumors often have an umbilicated and a lobulated appearance, particularly when they protrude above the capsule of the surrounding liver. The cut surface of the tumors varies from white to gray-white to yellow-brown. The borders of the lesions are generally well delineated from the adjacent hepatic parenchyma, although the border is frequently irregular. Areas of necrosis, characterized by softening of the tissue and reddish discoloration, can be found in the central regions of nodular tumors as well as in focal areas of large single neoplasms. Cystic areas containing yellow-brown viscous fluid may be randomly distributed throughout the Cholangiocarcinoma. The tumors are composed of cells that retain a resemblance to biliary epithelium. Characteristically, well-differentiated carcinomas are organized into a tubular or acinar arrangement. In less differentiated neoplasms, some acinar arrangements can be detected among solid masses of neoplastic cells. Poorly differentiated carcinomas are composed of packets, islands or cords, and areas of squamous differentiation can occur. The epithelial components of the neoplasms are usually separated by fibrous connective tissue. The amount of connective tissue varies among tumors, but an abundant deposition of collagen, termed a scirrhous response, is relatively common and is responsible for the firm texture of these neoplasms. The margins of cholangiocarcinomas are characterized by multiple sites of local invasion by tumor cells of surrounding hepatic parenchyma. Multiple sites of hepatic necrosis are also common in the adjacent parenchyma.

Metastasis to extrahepatic sites is common, particularly to the adjacent lymph nodes of the cranial abdomen, lungs, or by seeding into the abdominal cavity. Metastasis into the peritoneal cavity can produce variably sized nodules within the mesentery and on the serosal surface of the abdominal viscera.

Carcinoids

Carcinoids are uncommon tumors. They are believed to arise from neuroendocrine cells that lie within the biliary epithelium or possibly the hepatic parenchyma. They can form within the intrahepatic or extrahepatic biliary system. Often they form a single mass, but multiple nodules can occur, probably secondary to intrahepatic metastasis. Cells tend to be small, elongated, or spindle-shaped and form ribbons or rosettes and the tumors are highly vascular. Immunohistochemical detection of neuroendocrine markers, such as chromogranin A or neuron specific enolase, may be used to confirm the diagnosis in some cases, but there are no definitive markers.

Miscellaneous primary mesenchymal neoplasms of the liver

Primary neoplasms can arise from any of the normal cellular constituents of the liver, including mesenchymal neoplasms derived from the liver’s connective tissue, including fibrosarcoma, leiomyosarcoma, and osteosarcoma (probably secondary to another mesenchymal tumor initially) and endothelium (hemangioma and hemangiosarcoma). Primary hepatic hemangiosarcoma is
well recognized in dogs, although it is a relatively uncommon site of origin for this neoplasm as compared with the skin and spleen. Primary mesenchymal neoplasms of the liver must be distinguished from metastases; the presence of disseminated masses throughout the liver is more typical of metastatic sarcomas than of primary hepatic sarcomas.

**Metastatic neoplasms**

The liver is one of the two most common sites for metastatic spread of malignant neoplasms, a distinction shared with the lung. A complete necropsy and medical or surgical history are necessary to distinguish metastatic neoplasms from primary neoplasia of the hepatobiliary tissue. It is important, therefore, when evaluating a neoplasm within the liver to determine if a neoplasm is present at some extrahepatic site that might be the primary neoplasm. The animal’s medical history should also be reviewed to determine if masses have been removed previously.

Some metastatic neoplasms have a typical appearance within the liver; for example, melanomas frequently are black because of the presence of melanin, and hemangiosarcomas are usually dark red to brown because of blood. Malignant lymphoma is the most common metastatic neoplasm found in the liver of most, if not all, species and hematopoietic neoplasms, such as lymphoma and the myeloproliferative disorders, can diffusely expand the liver and can have diffuse infiltrative and nodular variants. Thus producing hepatomegaly and an enhanced lobular pattern on the cut surface, or they may have a nodular appearance. This characteristic appearance of diffuse involvement is attributable to centrilobular hepatocellular degeneration because of anemia in both lymphoma and myeloproliferative disorders and because of the specific location of accumulations of neoplastic cells; locations include portal and periportal for lymphomas and sinusoidal for myeloproliferative disorders. Metastatic carcinomas often have an umbilicated appearance, similar to that seen with cholangiocellular carcinomas, but umbilication is rarely a feature of sarcomas.