

EMERGING NEWER LIVER CONDITIONS TO BE AWARE OF IN THE DOG

David C. Twedt, DVM; DACVIM
Colorado State University

Several hepatobiliary disorders have recently come under increased awareness in dogs. Understanding these specific conditions is essential in the diagnosis and management of canine liver disease.

VACUOLAR HEPATOPATHY

Hepatic vacuolar change is a common histological diagnosis in dogs but not cats. When we reviewed 150 consecutive liver biopsies performed at Colorado State University approximately 12% of the cases had predominately a vacuolar hepatopathy (VH) as the major histological finding. By definition according to the WSAVA Liver Standardization Group VH refers to a reversible parenchymal change that is characterized by swollen hepatocytes with clear cytoplasm due to glycogen without displacement of the nucleus from the center. The distribution and the extent of the lesion can vary being either diffuse, zonal, or involve individual cells. VH is a relatively easy histological diagnosis to make however Periodic acid Schiff (PAS) staining with or without diastase can be used to demonstrate glycogen accumulation. Vacuolated hepatocytes can also result from fat accumulation secondary to abnormal fat metabolism and is referred to as hepatic steatosis or lipidosis. Hepatic steatosis is a distinct histological vacuolar classification associated with abnormal fat metabolism and will not be discussed in this chapter.

VH in dogs is most often associated with hyperadrenocorticism (HAC). The dog is particularly sensitive to the effects of glucocorticoids that both induce serum alkaline phosphatase (ALP) steroid isoenzyme activity and causes hepatic glycogen accumulation. (see chapter Evaluation of Elevated Alkaline Phosphatase in Evolve). Congenital glycogen storage disorders, breed specific disorders, hepatic nodular hyperplasia and a variety of stress-associated secondary diseases are conditions that can cause this typical hepatic vacuolar changes. In a large study of 336 histological liver specimens having VH (defined as making up greater than 25% of the hepatocytes) were retrospectively reviewed for an underlying etiology (Hill et al., 2006). The authors report 55% of the cases were associated with either endogenous or exogenous glucocorticoids with the remaining 45% having no known glucocorticoid exposure. Most all of the dogs with no glucocorticoid exposure had other identifiable concurrent illness. Conditions such as renal, immune-mediated, cardiac, hepatic, gastrointestinal disease, or neoplasia accounted for many cases. The author's hypothesis was that stress-induced hypercortisolemia associated with acute or chronic illness likely contributed to the development of the VH. A second *in vivo* study showed that by experimentally inducing a chronic four to five-fold elevations in plasma cortisol concentrations to simulate a stress-like state in normal dogs inhibited non-hepatic glucose utilization and increased hepatic gluconeogenesis and glycogen formation through enhanced substrate delivery to the liver.

Idiopathic Vacuolar Hepatopathy. There is a subset of dogs having elevations in serum alkaline phosphatase and excessive hepatic glycogen accumulation that do not have evidence of either a stress induced illness, evidence of HAC based on cortisol testing, a history of recent glucocorticoid administration or have a specific hepatic disease. These dogs are referred to as having an idiopathic vacuolar hepatopathy (IVH). They generally have no clinical signs and are usually identified during investigation of unexplained elevations in serum alkaline phosphatase (ALP) found on a routine health

screen. Several theories have been put forward as to the cause of IVH. Some believe adrenal progestagens; most likely increases in 17-hydroxyprogesterone and progesterone are responsible as these changes as they are frequently identified to be abnormal when a commercial adrenal steroid panel is performed. However, critical evaluation and validation of the adrenal steroid panel (17-hydroxyprogesterone, progesterone, estradiol, testosterone and androstenedione) is as yet still lacking and a direct association has not been made. Because the VH changes are typical of glucocorticoid excess it is entirely possible that a yet to be identified adrenal steroid could be responsible for the VH. Obviously future research is necessary to delineate this syndrome and the relationship to adrenal steroids.

Scottish terriers are also reported to have a breed-specific syndrome associated with a VH and elevated serum ALP. These affected dogs generally have no clinical signs. The authors found that the elevated ALP was predominately the corticosteroid isoform and following ACTH stimulation test in conjunction with an adrenal steroid panel found increases in one or more non-cortisol steroid hormones. The authors conclude that affected Scottish terriers have a type of hyperadrenocorticism on the basis of exaggerated adrenal hormone response. We have also observed similar non-cortisol steroid hormone increases in Scottish terriers but also in Scottish terriers without VH or increases in ALP adding more confusion to this syndrome. The reader should refer to Chapter 51, Occult hyperadrenocorticism: Is It Real? for further information concerning adrenal steroids.

Dogs with IVH generally have no clinical signs. They are usually identified serendipitously on a biochemical profile identifying elevations in serum ALP concentrations that subsequently initiates a diagnostic work-up. Most affected dogs are middle-aged or older at the time of diagnosis. There does not appear to be a breed or sex predisposition other than the syndrome described above in the Scottish terrier. A small percent of dogs may have reported polyuria and polydipsia (PU/PD) but the other signs typical of HAC are generally absent. The work up of the asymptomatic dog having an IVH usually begins after the identification of an elevation in serum ALP. The ALP increases are often 5 to 10 times normal concentrations; the other liver enzymes are usually normal or there are occasional mild elevations in alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT). Marked elevations in liver enzymes other than ALP is not typical of this syndrome and if present other types of liver disease should be investigated. The work-up should first rule out common causes for an elevated ALP such as drug administration (including topical or systemic steroids, phenobarbital, or herbal medications), cholestatic liver disease, or bone disorders. Next adrenal testing (ACTH stimulation or low dose dexamethasone suppression) would be prudent to perform to eliminate possibility of HAC. Determining the percent of ALP steroid isoenzyme is generally not helpful. Dogs with IVH will have predominately a steroid-induced ALP isoenzyme but this is neither specific for HAC or IVH and other non-adrenal illness may also have similar increases in the steroid-induced ALP isoenzyme. Basic tests of liver function tend to be normal however the author has seen a few cases having very mild elevations in serum bile acids. Abdominal ultrasound of the liver is helpful to rule out hepatic nodular hyperplasia, occult hepatic neoplasia or cholestatic disorders that all could be differentials for an elevated ALP. Affected IVH dogs generally have an enlarged uniformly hyperechoic liver with rounded borders. Adrenal glands are generally normal. Fine needle aspiration of the liver with cytology supports a diffuse vacuolar change. A PAS stain of the cytology sample can help confirm the presence of hepatic glycogen. A liver biopsy confirms diffuse vacuolar change but is rarely necessary. I generally make the diagnosis of IVH based on the above

diagnostic findings and after exclusion of HAC, drugs, hepatic nodular hyperplasia, hepatic neoplasia or cholestatic liver disease.

At this time I believe adrenal sex steroid panel testing for most cases is not necessary for two reasons; first, our inability to adequately interpret the tests results and second, most all IVH dogs are generally asymptomatic and information obtained from the testing offers little important diagnostic or therapeutic information. Several labs offer adrenal hormone analysis and currently the most extensive adrenal steroid hormone profile is offered by the Clinical Endocrinology Laboratory at the University of Tennessee. The protocol for running the test is identical to that for a standard ACTH stimulation test.

Both proteinuria or hypertension are occasionally identified in cases of IVH and the affected dogs should be periodically monitored for these complications and if identified, managed appropriately. Dogs with IVH are also thought to have an increased risk for developing biliary mucoceles and there is also some anecdotal evidence to suggest that some Scottish terriers with VH are at an increased risk of development of hepatic neoplasia (hepatocellular adenoma or carcinoma). Consequently it would be prudent to monitor IVH dogs from time with an ultrasound of the liver and biliary system.

The management of IVH is controversial at best and there are no studies critically evaluating therapy for this syndrome. I believe that specific therapy is unnecessary unless complicating factors such as hypertension, proteinuria or significant PU/PD exist. Problems associated with therapy arise from the fact we do not know what the endpoint of therapy should be; is it normalization of adrenal hormones, return of ALP into the normal range or histological resolution of the VH? There are anecdotal reports of dogs with IVH being successfully treated using low doses of mitotane and monitoring clinical parameters and measuring adrenal steroid concentrations including cortisol to assure hypoadrenocorticism does not result. Trilostane often shows a similar clinical response however concentrations of 17-hydroxyprogesterone and progesterone are frequently higher following this therapy. Anecdotal reports of clinical improvement in dogs having IVH using either of the therapy does suggest abnormal adrenal steroid production may be involved in the pathogenesis of this syndrome. However these treatments beg the question if therapy is warranted due to the expense of medication and monitoring and the potential complications associated with the therapy alone. Until more is known about this syndrome this author can't recommend specific adrenal therapy unless significant clinical findings would warrant a trial therapy. Alternative therapies suggested include melatonin and flax seed products. Melatonin has been shown to decrease sex hormone concentrations in normal dogs. It is reported to be beneficial in some dogs with alopecia X syndrome, and has also been suggested for IVH. Doses of 3 mg/15 kg q 24h PO has been recommended however there is no published data showing effectiveness for dogs with IVH. Flaxseed hull products with lignans have also been suggested because they compete with estradiol production but again there is no reported evidence of benefit for IVH syndrome.

Liver support therapy using products such as s-adenosylmethionine (SAME), the milk thistle products, or other antioxidants may have some beneficial effects. One study showed dogs given glucocorticoids and treated with SAME failed to show a decrease in serum ALP or amount of VH but did have improvement in hepatocyte oxidative status through increased glutathione concentrations. The above products are generally safe for liver support but will unlikely have any effect in the resolution of IVH.

HEPATIC NODULAR HYPERPLASIA

This is a benign process causing an increase in hepatic values and histomorphologic changes that include macroscopic or microscopic hepatic nodules containing vacuolated hepatocytes. Liver function remains unchanged. Grossly, the appearance may be suggestive of chronic hepatitis or neoplasia. The etiology is unknown but appears to be an aging change in dogs; most of those affected are greater than 10 years of age. Laboratory findings include an ALP increase (mean ALP ~ 600 IU/L), but some may have mild increases in ALT and AST concentrations as well. Ultrasound may be normal or may demonstrate larger nodules (many can be only microscopic and not observed on ultrasound). Biopsy confirms the diagnosis, however a wedge section is preferred. A needle aspirate or needle biopsy may only demonstrate show a vacuolar hepatopathy There is no specific therapy and it does not progress to a neoplastic process.

GALLBLADDER MUCOCELE

To date over 130 cases of gallbladder mucocele have been documented in the literature. A gallbladder mucocele is a condition that is described as an enlarged gallbladder with immobile stellate or finely striated patterns of mucoid material within the gallbladder lumen detected with ultrasound. The changes described often result in biliary obstruction or gallbladder perforation and peritonitis. Smaller breeds and older dogs are overrepresented. Shetland sheepdogs and Cocker Spaniels are most commonly affected. Most dogs are presented for nonspecific clinical signs such as vomiting, anorexia and lethargy. Abdominal pain, icterus and hyperthermia are common findings on physical examination. Most have serum elevations of total bilirubin, ALP, GGT and variable ALT although some dogs are asymptomatic and a mucocele is diagnosed as an incidental finding on abdominal ultrasound. The Shetland sheepdogs tend to have hyperlipidemia and a genetic defect in the MDR 3 hepatobiliary transporter gene involved phosphocholine transport into the bile. It is thought that the lack of phosphocholine in the bile may predispose to mucocoele formation. Risk factors identified in mucocele cases include endocrine disease (hypothyroidism, Cushing's disease) and idiopathic vacuolar hepatopathy (progesterones) and high fat diets.

Gallbladder mucoceles appear ultrasonographically as an immobile accumulation of anechoic-to-hypoechoic material characterized by the appearance of stellate or finely striated bile patterns (wagon wheel or kiwi fruit appearance). This should be differentiated from biliary sludge by the absence of gravity dependent bile movement; the mucocele is non-movable. The gallbladder wall thickness and wall appearance are variable and nonspecific. The cystic, hepatic or common bile duct may be normal size or dilated suggesting biliary obstruction. In one series, loss of gallbladder wall integrity and gallbladder rupture was present in 50% of the dogs and positive aerobic bacterial culture was obtained from bile in a majority of these dogs³¹. Gallbladder wall discontinuity on ultrasound indicated rupture whereas neither of the bile patterns predicted the likelihood of gallbladder rupture. Cholecystectomy is the treatment for mucoceles. There are reports of resolution of mucoceles using ursodeoxycholic acid (ursodiol) but even with a few cases reported to improve the recommendation is surgery in the sick dog³². Ursodeoxycholic acid is thought to upregulate the MDR 3 gene that may be the cause of mucocele production in some dogs.

Mucosal hyperplasia is present in all gallbladders examined histologically but infection is not present with all cases, suggesting biliary stasis and mucosal hyperplasia as the primary factors involved in mucocele formation. Based on information to date, the recommended course of action with an immobile ultrasonographic stellate or finely striated bile gallbladder with clinical or biochemical signs of hepatobiliary disease is a

cholecystectomy. A mucocele is reported the most common cause of a gallbladder perforation. Following cholecystectomy and recovery of postoperative period the prognosis is excellent especially when the liver enzymes are normal. Mortality rates are reported to be in the 20% range and some may persist in having liver disease with elevated liver enzymes.

PORTAL VEIN HYPOPLASIA

Portal vein hypoplasia (PVH) also referred to as microvascular dysplasia (MVD) is a common syndrome associated with abnormal microscopic hepatic portal circulation. It is thought that PVH is 15 to 30 times more common than a congenital portosystemic shunt (PSS). Hepatic PVH has been suggested as the terminology by the WSAVA Liver Standardization Group that may better reflect the etiology of this condition. It is believed that the primary defect in affected dogs is the result of hypoplastic small intrahepatic portal veins. This condition is thought to be a defect in embryologic development of the portal veins. With a paucity in size or presence of portal veins there is a resultant increased arterial blood flow in attempt to maintain hepatic sinusoidal blood flow. The hepatic arteries become torturous and abundant in the triad. Sinusoidal hypertension occurs under this high pressure system. Lymphatic and venous dilation results with opening up of embryologic sinusoidal vessels and thus acquired shunts develop to transport some of the blood to the central vein thus by-passing the sinusoidal hepatocytes. This results in abnormal hepatic parenchymal perfusion and lack of normal trophic factors bathing the sinusoids causing hepatic atrophy. With portal shunting of blood increased iron uptake also occurs that results in hepatic iron granuloma formation. Ascites or portal hypertension generally do not occur in this condition.

Because similar histological changes occur in dogs having PVH and PSS (ie., hepatic hypoperfusion) the diagnosis can be confusing. If an intrahepatic or extrahepatic macroscopic shunt is not observed then PVH becomes the probable diagnosis. Angiography or transcolonic portal scintigraphy fails to demonstrate macroscopic shunting in this condition. Often a needle biopsy is not sufficient to provide enough portal areas to make the diagnosis, and consequently a wedge or laparoscopic biopsy may be necessary.

The condition that was first described in Cairn terriers and now is felt to occur in other breeds of dogs. Yorkshire Terriers and Maltese may be over represented. Animals show no outward clinical signs and are usually identified because of elevated liver enzymes (ALT). All patients have abnormal serum bile acid concentrations (usually moderate elevations) but generally they are less than 100 $\mu\text{mole/L}$. It is reported PVH dogs have normal protein c concentrations while PSS dogs have concentrations less than 70% normal. There is no specific therapy. Some suggest antioxidants (SAMe, milk thistle). The long-term prognosis is uncertain because of lack of experience with this relative new disease. There may be a small number of dogs developing portal hypertension over time.

HEPATOCUTANEOUS SYNDROME

Hepatocutaneous syndrome, better known as superficial necrolytic dermatitis or metabolic dermatosis is an uncommon disease observed in middle aged to older dogs. The skin lesions have characteristic histological changes (superficial necrolytic dermatitis or necrolytic migratory erythema) and when combined with the hepatic changes typify this syndrome. The liver has mistakenly been described by some as cirrhotic because of the nodular appearance of the liver. The hepatic changes are best described as an idiopathic hepatocellular collapse with nodular regeneration. Changes are generally devoid of major inflammation. The hepatic nodular regeneration consists of vacuolated hepatocytes. To

date the pathogenesis of the hepatic disease is still controversial. In humans other types of liver disease have been noted to produce the similar cutaneous lesions however the hepatocellular collapse described in the canine hepatocutaneous syndrome has not been reported. It is not known if the liver dysfunction is the major mediator of the necrolytic skin lesions or whether another metabolic disease produced both the skin and hepatic lesions. Affected dogs almost all have pronounced reductions in amino acid and albumin concentrations. Some authors believe this condition to be the result of exaggerated amino acid catabolism. Uncommonly some dogs and humans have hyperglucagonemia secondary to a glucagon-secreting tumor. Diabetes mellitus occurs in some dogs. Recently hepatocutaneous syndrome has also been associated with chronic long-term phenobarbital therapy.

Most dogs are presented because of the skin disease. Abnormal liver enzymes are identified and in most, ALP and bile acids are increased. The albumin is typically below normal and almost every affected dog is hypoaminoacidemia. The liver has a characteristic ultrasound appearance looking like "Swiss cheese" due to the hypoechoic nodules. It is thought that the necrolytic skin lesions are directly related to the hypoaminoacidemia. The hypoaminoacidemia may be responsible for the hepatic changes as well. This is supported in part by observations that dogs fed a protein deficient diet for prolonged periods develop hypoalbuminemia and hepatic changes that resemble hepatic changes described in the hepatocutaneous syndrome, however skin lesions were not observed. The importance of hypoaminoacidemia in this disease is further supported in that administration of intravenous amino acid solutions transiently improved the lesions in many but not all dogs. The cause of the amino acid deficiency is unknown. The affected dogs appear to have been feed adequate protein content diets. The reported prognosis for this disease is grave and invariably most succumb either due to liver dysfunction or to the severity of the skin lesions, or both.

Our current therapy includes administration of intravenous amino acid solution. We give approximately 500 ml of Aminosyn™ (10% solution, Abbott) over 8-12 hours. If given too fast, hepatic encephalopathy can occur. Repeated infusions are given weekly. If after four weekly amino acid infusions and if there is no improvement it is unlikely the patient will respond to therapy. Some dermatologists suggest that daily infusions of amino acids for the first week results in a quicker response. With a positive response repeated the amino acid infusions are given as needed. In addition, we generally treat the patient with a dietary protein supplement of egg yolks (as an amino acid source) and other protein supplements. Additional support includes antibiotics if a secondary skin infection exists, omega 3 fatty acids, ursodeoxycholic acid, vitamin E and/or zinc.

DRUG ASSOCIATED LIVER DISEASE

Drugs are the most common known cause of ALF in dogs and cats. Drugs can affect the liver in one of two ways. First, they may have a direct toxicity to hepatocytes or becomes metabolized to a toxic compound that then causes damage. This first classification is referred to as a direct hepatotoxin and is dose related and reproducible. An example would be acetaminophen poisoning and CCNU therapy. More common however are drugs associated with an idiosyncratic drug reaction. Idiosyncratic drug reactions are unpredictable and not dose related but most often associated with abnormal or aberrant metabolism of the drug to a toxic compound. It should be noted however any drug metabolized by the liver has the potential to be a hepatotoxin. The common incriminators causing an idiosyncratic reaction include the NSAIDs, trimethoprim-sulfa, lysodren, ketoconazole (and other antifungals), and diazepam (in cats) to name but a few.

At Colorado State University we reported on a series of dogs developing acute liver toxicity associated with carprofen. The toxicity was idiosyncratic occurring in possibly 1 out of 10,000 dogs. We have also more recently identified toxicity associated with azathioprine. Some herding breed dogs lack p-glycoprotein that plays an important role in metabolism of many drugs. Thus it is not surprising that lack of P-glycoprotein, which occurs in many herding-breed dogs leads to increased susceptibility to drug toxicosis.

Clinical signs may be silent or be severe based on the amount of liver damage (generally necrosis). The clinicopathologic changes reflect the extent of necrosis and loss of hepatocyte numbers. The hepatic transaminases (ALT and AST) are released when the cell membrane is damaged and the cytosol enzymes leak out. A marked increase in AST to ALT ratio suggests more severe hepatocellular damage.

When giving a patient a potential hepatotoxic drug it is prudent to follow up on liver enzymes at a later date. For example when I give a NSAID I get pretreatment blood work and recheck an ALT 7-14 days after starting the therapy. With idiosyncratic drug reactions there is no evidence that increased liver enzymes from a pre-existing liver condition will increase the frequency of toxicity. With direct hepatotoxins like CCNU or amiodarone it might be wise to prescribe liver support suggested below.

The next step is to prevent further drug-associated hepatocyte damage is to provide an environment for optimal hepatic function. There is considerable evidence showing that free radicals are generated in acute liver damage and participate in the pathogenesis of liver injury. Free radicals are molecules with an unpaired electron that form by the injurious effects of certain drugs or various other toxic agents or events. Free radicals, if not inactivated, damage cellular macromolecules via lipid peroxidation and thus participate in cellular injury when produced in excess. Depletion of antioxidants primarily glutathione parallels hepatic damage. N-acetylcysteine (NAC, Mucomyst™) is thiol (SH) donor and promotes the production of glutathione. Glutathione is the most important detoxifier of toxic cellular xenobiotics. There is also evidence that NAC protects against hepatic ischemia-reperfusion damage possibly by inhibiting Kupffer cell function. Further NAC has beneficial effects on liver blood flow, oxygen extraction, and the formation of non-glutathione products that protect against cell injury. Experimentally NAC has protective effects against aflatoxin damage as well. The suggested dose for NAC is 140 mg/kg IV followed by 70 mg/kg IV bid or tid for one to three days. The injectable NAC should be diluted 1:4 in 5% dextrose and water and given slowly over 30 minutes to 1 hour. When vomiting has resolved NAC therapy can be switched to oral medications. Oral S-Adenosylmethionine (SAME) also protects against liver damage in dogs and cats by increasing hepatocyte glutathione concentrations being a SH donor. It also acts as a methyl donor and enzyme activator for key reactions that maintain membrane structure and function. Reports show protection against acetaminophen toxicity in the dog and cat. SAME is given orally at the dose of 20 mg/kg bid or daily. SAME in combination with milk thistle products is commercially available and would be of benefit as well.

The use of other antioxidants is warranted in management of the liver disease including vitamin E and milk thistle or its by-products. Vitamin E, d-alpha tocopherol, functions a major membrane bound intracellular antioxidant, protecting membrane phospholipids from peroxidative damage when free radicals are formed. Vitamin E is shown to protect against the effects of copper, bile acids and other hepatotoxins. A suggested vitamin E dose is 50 to 400 IU a day. Milk thistle has been used for centuries as a natural remedy for diseases of the liver and biliary tract. Silymarin the active extract consists of bioflavonolignans that have been reported to work as antioxidants, scavenging

free radicals and inhibiting lipid peroxidation. In a number of human clinical studies on patients having either acute or chronic liver disease has provided mounting evidence of the benefit of milk thistle. These studies must be interpreted with care because of the variable experimental design and limited number of cases. One canine study showed that dogs poisoned with amanita mushrooms that were treated with milk thistle had less clinical signs and complete survival while one-third of dogs in the untreated group died. Due to the lack of standardization of milk thistle preparations it is difficult to provide an appropriate dosage. Suggestions have included 50-250 mg/kg bid. Milk thistle is reported to have an extremely low toxicity in humans and animals and has been used extensively in clinical patients with little concern for side effects. It appears to have a synergistic effect with vitamin E.

The remainder of the therapy is only symptomatic treating any complications associated with liver damage.

Some drugs associated with liver toxicity.

Acetaminophen	Arsenicals
Ketoconazole	Sulfonamides
Halothane	Carprofen (NSAIDs)
Griseofulvin	Itraconazole/ketoconazole
Mitotane (lysodren)	Trimethoprim-sulfa
Diazepam	Anabolic steroids
Doxycycline	Anticonvulsant drugs
Azathioprine	Antineoplastic drugs
Amiodarone	CCNU