Gallbladder Mucocele in a Dog

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Introduction

This paper describes the clinical signs, diagnosis and treatment of a gallbladder mucocele in a dog. Gallbladder mucocele (GBM) formation is a unique and emergent disease syndrome of dogs characterized by an insidious accumulation of thick, immobile, and viscous bile and mucus within the gallbladder lumen.[1,2] The etiology of GBM development is unknown.[2,3,4] Factors predisposing to GBM formation include a middle to geriatric age; hyperlipidemia/hypercholesterolemia associated with pancreatitis, nephrotic syndrome, endocrinopathies, or idiopathic causes; gallbladder dysmotility; and cystic hyperplasia of the gallbladder leading to increased mucous production.[5] Once considered a rare postmortem finding, GBM has emerged as one of the most commonly recognized causes of gallbladder disease in dogs over the last decade.[2] The apparent increase in the diagnosis of GBM may be due to the gradual incorporation of ultrasonography into clinical practice, allowing visualization of the distinct ultrasonographic appearance of a GBM in combination with historical information, physical examination and serum biochemistry findings.[5,6,7] At the time of diagnosis of GBM, elective surgery secondary to clinical signs or emergency surgery secondary to gallbladder compromise or possible rupture remains the mainstay of treatment, often with resolution of clinical signs and serum chemistry abnormalities.[5]

Bile, produced by sheets of hepatocytes, consists of bilirubin glucuronide, which is a water

soluble product of hemoglobin degradation, bile salts, cholesterol, phospholipids, and plasma electrolytes.[8] The production of bilirubin originates from two primary functions: the majority of bilirubin (65-75%) comes from the breakdown of hemoglobin taken from senescent erythrocytes in the reticuloendothelial system of the spleen and bone marrow, while the remaining bilirubin (25-35%) is produced from the turnover of hepatic heme and hemoproteins.[9] Most of the bilirubin produced in the liver is released into the circulation in the unconjugated form.[9] Unconjugated bilirubin is very tightly bound to albumin, and conjugated only slightly less so, so that very little circulating bilirubin is free.[9] Hepatic bilirubin uptake involves both the unbound and albumin-bound bilirubin.[9] After hepatic conjugation, the bilirubin is carried by active transport against a concentration gradient into bile. Virtually all the bilirubin excreted into bile is conjugated, under normal conditions.[9] Urobilinogen is formed in the colon by deconjugation of bilirubin, and about 10 -20% of what is formed is reabsorbed into the circulation.[9] About 95% of that is reabsorbed from the portal blood by the liver, and returned unchanged to the intestine in the bile.[9] Whatever escapes the liver is rapidly cleared by the kidneys.[9]

Bile is collected in the bile canaliculi which unite to form the biliary ductules.[10] The biliary ductules continue to form interlobular ductules, which form the portal triad with branches of the hepatic artery and portal vein.[10] Interlobular ductules anastomose to form larger interlobular ducts, which unite into lobar intrahepatic ducts.[10] Lobar intrahepatic ducts give rise to extrahepatic ducts including variably numbered hepatic ducts.[10] The hepatic ducts exit the liver from its lobes and, in most domestic species, join the cystic duct that empties the gallbladder.[8] The bile duct is formed by the joining of the cystic duct with two or more hepatic

ducts and enters the duodenum at the major duodenal papilla.[10]

The gallbladder, a pear-shaped vesicle, is found in the right cranial abdomen situated in the gallbladder fossa between the quadrate liver lobe medially and the right medial liver lobe laterally.[10] The main function of the gallbladder is the storage and modification, via concentration, acidification, or addition of mucin or immunoglobulins, of bile produced in the liver.[5,7,11] The gallbladder wall is comprised of five histologically distinct layers including, from innermost, the epithelium, submucosa, tunica muscularis externa, tunica serosa, and tunica adventitia.[11] The normal gallbladder mucosa consists of mucosal folds that anastomose to form polygonal structures lined by columnar epithelium and sub-epithelial glands where mucin secretory epithelial cells are concentrated.[12] The gallbladder epithelium plays a key role in the transport of water and electrolytes, acidification of bile, and reabsorption of cholesterol and other bile lipids.[12] The integrity and functionality of the epithelium is protected by secretions of mucins that serve as a barrier against exposure to bile.[12] Gallbladder emptying is triggered by ingesta arriving in the proximal small intestine and is mediated postprandially by cholecystokinin.[13,14] The sole vascular supply to the gallbladder is the cystic artery which is derived from the hepatic artery, making the gallbladder susceptible to ischemic necrosis if these vessels become compromised.[5]

The etiology of GBM is not completely understood but is suspected to be complex and multifactorial.[15] Any condition or disease that results in cholestasis, which is defined as a reduction in bile volume or impairment of gallbladder emptying, is thought to play a role in the development of cholecystitis and GBM formation through prolonged exposure of the gallbladder epithelium to concentrated bile acids, initiating or aggravating epithelial damage.[9,13]

Cholestasis can result from various mechanisms.[9] In addition to increased intraluminal biliary pressure due to bile duct obstruction, cholestasis may occur due to abnormal bile acid concentrations, inhibition of bile flow that is not bile-acid dependent, or changes in canalicular membranes.[9] This results in overproduction of mucus and hyperplasia of the mucin secreting epithelial cells, a condition commonly known as cystic mucinous hyperplasia.[12,16] In a GBM, the normal mucosal folds become flattened with epithelial projections extending into the gallbladder lumen and replacement of sub-epithelial glands and mucin secretory cells with columnar epithelium occurs, perhaps indicating that GBM formation is not a consequence of glandular hyperplasia.[12] Large amounts of mucus may be observed from all epithelial cells.[12] Increased mucus production results in an increase in osmotic pressure which affects the distribution of water between the mucus and the epithelial layer.[12] This leads to a more concentrated mucus and eventual adhesion of the mucus to the epithelial surface, immobilizing the mucus layer.[12] Biliary sludge formation, gallbladder dysmotility, biliary stasis, mucus hypersecretion, and cystic mucinous hyperplasia may represent a continuum with formation of a GBM as the end stage of the disease process.[16]

Biliary sludge has been suspected to be a predisposing factor in the development of a GBM but the association between the development of biliary sludge and GBM formation remains unclear.[6,11,13] Biliary sludge is defined as the presence of gravity dependent, non-shadowing, echogenic material within the gallbladder lumen commonly seen on ultrasonographic examination.[16] The exact composition of gallbladder sludge has not been reported in dogs but, in people, sludge is an accumulation of cholesterol monohydrate crystals or calcium salt granules.[13] Historically, the presence of mild to moderate biliary sludge has been commonly

dismissed as an incidental finding.[16] However, recent studies have indicated that the presence of gallbladder sludge may not be a benign process.[14,16] Normal gallbladder emptying is thought to have a cleansing effect on the biliary tract through drainage of stored bile.[13] The presence of gallbladder sludge has been associated with gallbladder dysmotility and decreased gallbladder emptying.[13,16] Specifically, dogs with sludge occupying greater than 25% of the gallbladder lumen had larger overall volumes compared to dogs with sludge occupying less than 25% of the gallbladder lumen, a likely indication of decreased gallbladder emptying.[14]

Concurrent endocrinopathies, such as hyperadrenocorticism, hypothyroidism, and diabetes mellitus, have been suspected to play a role in GBM development. A clinician should have a heightened degree of suspicion of a GBM in dogs with preexisting hyperadrenocorticism or hypothyroidism that present with acute illness and typical biochemical changes.[17] Likewise, dogs diagnosed with a GBM may be screened for concurrent endocrine disease if a clinical suspicion is present.[17] One study found that dogs with hyperadrenocorticism were 29 times more likely to have findings of a GBM.[17] However, in another study, dogs received twice daily exogenous glucocorticoids for 84 days to experimentally simulate hyperadrenocorticism.[6] Gallbladder sludge was noted in both treated and untreated dogs with no significant difference between groups.[6] Specifically, all treated dogs had sludge at day 56 of the study, but 50% of non-treated dogs had sludge.[6] A change in bile pH may affect the solubility of various bile acids and their salts.[6] The state of iatrogenic hyperadrenocorticism did cause an increased but reversible pH and concentration of cytotoxic, hydrophobic, unconjugated bile salts within the bile which may precipitate increased mucin secretion and gallbladder dysfunction.[6] Increased risk of bacterial cholecystitis due to concurrent immunosuppression and alterations in

gallbladder motility that lead to the development of a GBM have also been suspected secondary to hypercortisolemia.[17]

No screening test for hyperadrenocorticism is 100% accurate.[18] A low-dose dexamethasone suppression test (LDDST) is the preferred screening test due to its high sensitivity (85-100%) and adequate specificity (44-73%) compared to other screening tests.[18] The test is performed by drawing an initial sample of blood followed by the administration of 0.01 mg/kg dexamethasone sodium phosphate intravenously (IV).[18] Additional blood samples are drawn at four and eight hours following the administration of the dexamethasone sodium phosphate and the cortisol levels are measured in each sample.[18] Adequate suppression of the hypothalamic-pituitary-adrenal axis and, thus, cortisol release from the adrenal glands is indicative of the absence of hyperadrenocorticism.[18]

Although positive or negative screening tests are considered the gold standard to confirm or eliminate the presence of hyperadrenocorticism, ultrasonography has been determined to be a useful primary screening modality to identify increased adrenal gland width in cases of pituitary-dependent hyperadrenocorticism (PDH), decreased adrenal gland width in cases of hypoadrenocorticism, and loss of normal cortical or medullary architecture in cases of adrenal neoplasia.[19] In a recent study, adrenal gland width, considered to be more representative of gross adrenal size compared to the adrenal gland length, was evaluated in small breed dogs weighing less than 10 kilograms; ultrasonographic characteristics of normal adrenal glands were compared to those of adrenal glands with PDH.[19] The median adrenal gland width in normal dogs was 0.42 cm while the median adrenal gland width in dogs with PDH was 0.63 cm.[19] The study concluded that a cut-off value between the width of normal adrenal glands and PDH in

small breed dogs was 0.6 cm, yielding a sensitivity and specificity of 75% and 94%, respectively, for detecting PDH.[19]

It must be stated that subjective assessment of adrenal gland size with ultrasound is not a definitive assessment in cases of PDH as ultrasound alone cannot confirm the functionality of the adrenal gland.[20] In a small number of cases, or if it is early in the disease process, the adrenal glands may measure normally.[20] Prominent adrenal glands may also be seen in dogs with non-adrenal illnesses secondary to stress and increased cortisol production.[20] The presence or absence of clinical signs associated with hyperadrenocorticism, such as polyuria, polydipsia, polyphagia, panting, a pendulous abdomen and hepatomegaly, and dermal abnormalities including alopecia, comedones, and hyperpigmentation, may be used in conjunction with ultrasonographic measurements of the adrenal glands to determine if an adrenal screening test is warranted.[21] Due to polydipsia, approximately 85% of dogs with Cushing's syndrome will have a urine specific gravity less than 1.020.[21]

Occult or atypical hyperadrenocorticism may be considered when dogs have clinical signs of hyperadrenocorticism, no sex hormone adrenal tumor, and adrenal testing is not consistent with hyperadrenocorticism.[18] A sex hormone panel following an adrenocorticotropic hormone stimulation test may be considered to assess for atypical hyperadrenocorticism. Interpretative caution must be given to the results of this test as dogs with and without non-adrenal illness may have elevated levels of sex hormones.[18] In general, if the clinical picture does not fit testing for classic hyperadrenocorticism, it does not fit testing for atypical hyperadrenocorticism.[18]

Hypothyroidism may also play a role in GBM development but is represented to a lesser degree

than hyperadrenocorticism.[17] Thyroxine was found to allow relaxation of the sphincter of Oddi, a circular band of muscle tissue located at the end of the biliary tree that controls the flow of bile and pancreatic secretions into the duodenum.[17] Delayed bile emptying was found in both humans and rats with hypothyroidism due to low concentrations or absence of thyroxine.[17] Subsequent biliary stasis and prolonged retention of bile may result in modification or concentration of the bile, secondary epithelial irritation and mucinous hyperplasia, and a GBM.[17] The odds of GBM development were no different in dogs with or without diabetes mellitus.[17] Hypothyroidism is diagnosed by documenting the presence of low serum levels of total thyroxine and free thyroxine.[22] The thyroid-stimulating hormone level is often high due to the loss of negative feedback of total thyroxine on the pituitary gland.[22] Low thyroxine levels may also be seen with nonthyroidal or concurrent illness.[23]

Dyslipidemias, such as hypercholesterolemia and hypertriglyceridemia, are often seen in endocrinopathies; but breed-specific primary hyperlipidemia, specifically hypercholesterolemia in Shetland Sheepdogs and hypertriglyceridemia in Miniature Schnauzers, has been reported.[3] In a fasted state, hyperlipidemia is caused by a disturbance in the metabolism, either an overproduction or decreased removal, of the lipoprotein responsible for carrying hydrophobic fat, triglycerides and cholesterol, to and from tissue.[24] High blood concentrations of chylomicrons and very-low-density lipoprotein (VLDL) will appear as high plasma triglyceride levels, whereas high concentrations of high-density lipoprotein (HDL) and/or low-density lipoprotein (LDL) raise plasma cholesterol levels.[24] Hypertriglyceridemia is more clinically relevant than hypercholesterolemia.[24] Clinical signs associated with hyperlipidemia vary from mild abnormalities, such as diarrhea, vomiting, or abdominal pain, to more severe

presentations, such as pancreatitis, seizures, peripheral nerve paralysis, and behavioral changes.[24]

The relationship between GBM and hypercholesterolemia appears to be directly related to excessive excretion of cholesterol in the bile, perhaps as part of a catabolic escape pathway for cholesterol from the body, with subsequent oversaturation of the bile leading to the formation of biliary sludge and subsequent reduced gallbladder motility.[3,6] Hypercholesterolemia may also be associated with decreased bile excretion in cases of bile duct obstruction.[25] Postprandial- and cholecystokinin-stimulated gall bladder motility has been reported to be decreased in hypertriglyceridemic humans, with improvements in gall bladder motility following successful triglyceride-lowering therapy.[3,26] It is possible that hypertriglyceridemia may reduce gall bladder motility in dogs, resulting in prolonged exposure of the gallbladder mucosa to concentrated cytotoxic, hydrophobic bile acids.[3]

If a primary hyperlipidemia is suspected, secondary causes of elevated cholesterol and triglycerides should be ruled out first.[27] Endocrine diseases associated with hyperlipidemia, such as hypothyroidism, diabetes mellitus, and hyperadrenocorticism, should be definitively ruled out.[27] Other diseases associated with hyperlipidemia include nephrotic syndrome, cholestasis, and pancreatitis.[27] With treatment of a secondary cause, lipid abnormalities will usually improve or resolve.[27] If secondary causes are eliminated, primary hyperlipidemia may be verified after a confirmed 12-18 hour fast.[27] Additional diagnostic tests include a serum turbidity test, which provides an estimation of triglyceride content, and a refrigeration test, which can help delineate the lipid type.[27]

Treatment for a primary hyperlipidemia is recommended if the fasted triglyceride levels are greater than 500 mg/dl or cholesterol levels are greater than 750 mg/dl.[24,27] A realistic goal of therapy is to reduce the triglyceride concentrations to less than 400 mg/dl. Initial treatment consists of a diet change to a low fat food (less than or equal to 30 g/Mcal) with reevaluation in six to eight weeks.[24] If the patient is already on a fat-restricted diet, changing to an ultra-low-fat diet (less than 10 g/Mcal) formulated by a nutritionist is recommended.[24] Since hypertriglyceridemia caused by disturbances in the metabolism of endogenous VLDL lipoproteins may not fully respond to a low-fat diet, supplementation with fish oil containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can be added to the treatment regimen.[24,27] These omega-3 fatty acids may act by decreasing the production of VLDL; stimulating the activity of lipoprotein lipase, which hydrolyzes the triglyceride core of chylomicrons to free fatty acids and glycerol; decreasing the intestinal absorption of lipid; and increasing the secretion of cholesterol into bile.[27] The use of pharmaceutical lipid-lowering agents, such as gemfibrozil, niacin, or statin medications, should only be attempted in animals that have severe hypertriglyceridemia that cannot be improved by a low-fat diet and fish oil therapy.[24,27] These medications have adverse effects, primarily vomiting, diarrhea, abdominal pain, or abnormal liver function tests, and lack sufficient studies to determine the dose, duration or toxicity levels.[27] Chitosan, a natural polymer of glucosamine derived from the cell walls of some fungi and the exoskeleton of crustaceans, including shrimps, crabs, lobsters, and prawns, has been shown to have beneficial effects on serum lipid levels, including cholesterol and triglycerides.[28]

A genetic factor may also predispose Shetland Sheepdogs to GBM development.[7,29] As

mentioned earlier, bile is produced by hepatocytes and initially collected in the bile canaliculi.[10] Active transport of biliary solutes, such as bile salts, phospholipids, and cholesterol, is accomplished by a number of transporters located in the canalicular membrane.[29] The phospholipids, particularly phosphatidylcholine, reduce bile salt toxicity.[29] The gene adenosine triphosphate-binding cassette subfamily B member 4 (ABCB4) produces proteins that function as phosphatidylcholine translocators across the hepatocyte canalicular membrane into the biliary canaliculi lumen.[29] An insertion mutation in ABCB4 eliminates approximately one-half of the protein, thereby reducing the amount of bile phosphatidylcholine content and increasing the cytotoxicity of bile salts.[29] This mutation was found in 93% of affected Shetland Sheepdogs, but not present in 95% percent of unaffected individuals, in one study.[29] A subsequent study found that there was no statistically significant association between an insertion mutation in ABCB4 and the presence of GBM for all dogs combined or for Shetland Sheepdogs alone.[30]

Selected drug use has also been investigated for possible association with GBM formation, particularly in Shetland Sheepdogs.[2] Dogs with a histologic diagnosis of a GBM were 2.2 times as likely to have reported use of products containing imidacloprid, an insecticide that binds to and blocks nicotinic acetylcholine receptors.[2] Nicotine has been shown to decrease mucus transport, increase mucin secretion, alter mucus hydration, and increase the viscosity of mucus in the airway; its effects in the gallbladder epithelium have not been examined.[2] Prolonged activation or desensitization of nicotine acetylcholine receptors may interact with mechanisms suspected to underlie GBM formation.[2] Shetland Sheepdogs with a histologic diagnosis of GBM were 9.3 times more likely to have reported use of products containing imidacloprid when matched with control Shetland Sheepdogs.[2] This finding does not suggest that imidacloprid

could be a primary cause of GBM formation in dogs, but possibly be a contributing factor in GBM in Shetland Sheepdogs.[2] No association between other commonly used drugs, including ivermectin, milbemycin, nonsteroidal anti-inflammatory medications (NSAIDS), or joint supplements, and GBM was found.[2]

Cholestatic syndromes generally lead to hepatic injury, dilation of the bile duct, proliferation of ductules, and fibrosis with both conjugated and unconjugated hyperbilirubinemia.[9] With chronicity, irresolvable distension of the major bile ducts develops secondary to fibrosis.[5] Icterus or jaundice, synonymous terms used to describe when the level of hyperbilirubinemia is evidenced in the tissues (most notably the sclera, mucous membranes, and skin), are subjective physical examination findings and do not necessarily correlate with the degree of hyperbilirubinemia.[31] Icterus may be noted when the level of serum bilirubin reaches 2.0-4.0 mg/dl although icterus may not be present in acute disease.[31] Although the severity of hyperbilirubinemia may lead a clinician to suspect or prioritize different disease processes, the diagnostic approach should remain consistent in an icteric patient whether the patient has mild or marked hyperbilirubinemia.[31]

A minimum data base including a complete blood count, biochemistry profile, and urinalysis is generally obtained when a dog presents with the various clinical signs associated with hyperbilirubinemia.[31] These tests assist in the classification of the hyperbilirubinemia as prehepatic, hepatic, or posthepatic.[31] Additional diagnostics can then be tailored to the differential diagnoses with each class of hyperbilirubinemia.[31] For example, a moderate to severe regenerative anemia is most consistent with prehepatic hyperbilirubinemia secondary to hemolysis.[31] Changes in erythrocyte morphology, such as the presence of spherocytes or

acanthocytes, are also indications of prehepatic disease.[31] If an anemia is present, a saline agglutination test may be performed to assess for red blood cell autoagglutination.[32,33] This test is performed by combining one drop of anticoagulated blood from a tube containing ethylenediaminetetraacetic acid with a drop of saline on a microscope slide.[32,33] Microscopic evaluation of the saline-diluted red blood cells is necessary to differentiate autoagglutination from rouleax formation, the physiologic aggregation or stacking of red blood cells in vertebrates.[32] A direct antiglobulin test, or Coombs' test, detects erythrocyte-bound immunoglobulin and may be considered when the diagnosis of autoagglutination on a saline agglutination test is not obvious.[32] A microscopic evaluation of fresh blood smears by a clinical pathologist is often incredibly valuable in the detection of erythrocyte morphology changes or the identification of infectious diseases that may cause hemolysis.[31]

Hepatic hyperbilirubinemia may occur as the result of acute to chronic hepatic parenchyma disease, inflammation, or hepatic failure.[34] In veterinary medicine, acute liver failure is most commonly caused by hepatotoxin exposure, inflammatory or immune mediated disease, infectious organisms, infiltrative neoplasia, trauma, and hypoxic injury.[31,34,35] Ingestion of Amanitum spp. mushrooms, blue-green algae, sago palm and some drugs, such as sulfonamides, acetaminophen, and carprofen, may result in significant liver disease.[35,36] Copper-associated hepatitis, a form of chronic hepatitis, is thought to result from an inherited enzymatic defect in several breeds, especially the Bedlington Terrier, but also Dalmations, Labrador Retrievers, and West Highland White and Skye Terriers.[36,37] Copper qualification or quantification after hepatic biopsy is needed to diagnose elevated hepatic copper levels.[36] Hepatic copper concentrations in dogs with secondary copper accumulation generally fall in the

range of less than 750 mcg/g dry weight and copper accumulates in zone 1 (adjacent to the area of hepatic injury) of the liver; while breed associated hepatotoxicities generally have higher concentrations (> 750 mcg/g) and copper accumulation appears to begin in zone 3 of the liver (centrolobular).[38] The copper chelator d-penicillamine 10 to 15 mg/kg PO q12h per os (PO) is preferred for removing excess copper, and elemental zinc 10 mg/kg q12h PO can decrease gastrointestinal copper absorption; however, these two medications should not be used simultaneously.[36] Chronic hepatitis may eventually lead to hepatic fibrosis and cirrhosis with resultant hyperbilirubinemia.[36]

Leptospirosis, a zoonotic bacterial disease with a worldwide distribution, appears to be increasingly prevalent and usually leads to both hepatic and renal insults, resulting in polyuria, polydipsia with or without azotemia, oliguria, or anuria.[36,39] Other clinical manifestations may include fever, shivering, generalized muscle discomfort, conjunctivitis, uveitis, tachypnea or dyspnea secondary to leptospiral pulmonary hemorrhage syndrome, vasculitis, and disseminated intravascular coagulation.[39] Disease in dogs is caused primarily by Leptospira interrogans *(*serovars Icterohaemorrhagiae, Canicola, Pomona*,* and Bratislava in the United States) and Leptospira kirschneri *(*serovar Grippotyphosa in the United States).[39] Culture and polymerase chain reaction (PCR) detect pathogenic leptospires or their nucleic acid, respectively, and have potential utility early in the course of untreated infection when antibody assays are frequently negative and antimicrobials have not yet been administered.[39] They also can confirm active infection in animals with positive antibody test results that have a history of vaccination with leptospiral vaccines, because previous vaccination should not yield false positive results by these methods.[39] These tests may detect infection in dogs with chronic renal

or hepatic disease.[39]

Caution is recommended when handling dogs suspected of having leptospirosis.[39] Warning labels should be placed on cages of dogs that are leptospirosis suspects, and movement of the dog around the hospital should be minimized.[39] Gloves and a disposable gown should be worn when handling the patient, and either protective eyewear or a facemask used when cleaning areas of urine spillage.[39] Hand washing is suggested after handling the patient even with the use of gloves.[39] Frequent walking in a restricted, easy to clean area is necessary to avoid urine spillage; but, if spillage does occur, immediate cleaning and disinfection is needed.[39] If suspected, treatment for leptospirosis should not be delayed pending the results of diagnostic testing.[39] Doxycycline 5 mg/kg PO or IV q12h for two weeks is the recommended antibiotic for treatment of both suspected and confirmed leptospirosis, while ampicillin 20 mg/kg IV q6h is recommended if vomiting or other adverse reactions preclude the use of doxycycline.[39] Other potential infectious or parasitic agents that may result in hepatitis include infectious canine hepatitis (canine adenovirus-1), Clostridium piliformis, Escherichia coli, Toxoplasma gondii and trematodes.[35,36]

Aside from a GBM, posthepatic causes of icterus include cholecystitis, pancreatitis or pancreatic neoplasia, duodenitis or duodenal neoplasia, biliary neoplasia, or cholelithiasis.[31] These conditions may result in similar clinical signs and clinicopathologic abnormalities as hepatic causes of hyperbilirubinemia, and differentiation between the two causes of icterus can be challenging.[31]

Signalment, history and clinical signs associated with hyperbilirubinemia may offer the clinician

clues as to the presence of a GBM. Risk factors for GBM development may include older small to medium breed dogs with no sex predilection.[11] The average age at the time of diagnosis of GBM is nine to ten years of age.[1,11] Breeds associated with increased GBM development, aside from Shetland Sheepdogs, may include Cocker Spaniels, Miniature Schnauzers, Pomeranians, and Chihuahuas.[1,7,11] Clinical signs, which are similar to those often attributed to pancreatitis, are generally broad or non-specific and include vomiting, diarrhea, lethargy, anorexia, abdominal pain, icterus, and possible shock or death if bile peritonitis develops.[4,7,11,25] Approximately 23% of dogs with a GBM do not show clinical signs.[4]

If the patient is painful, the determination of the type and location of pain may allow the astute clinician to establish a more specific differential diagnosis list. Pain generally arises from somatic or visceral origins.[40] Somatic pain originates from the musculoskeletal or other peripheral systems, and tends to be distinct and well localized.[40] Visceral pain, or pain originating from the internal organs, is less well organized or distinct and may be reflected by distension, inflammation, or ischemia within the affected organs.[40] Numerous pain assessment methods have been extrapolated from human studies and may rely on both physiologic (objective) and behavioral (subjective) criteria.[40] Commonly used semi-objective acute pain scales include the simple descriptive scale and the numerical rating scale, where numbers from zero to four and zero to ten, respectively, are assigned to the degree of pain.[40]

Clinicopathologic abnormalities secondary to a GBM may vary between symptomatic and asymptomatic dogs and may include the following: a leukocytosis, with or without a left shift neutrophilia; increased liver enzymes, including alkaline phosphatase (ALKP), gamma-glutamyltransferase (GGT), alanine aminotransferase (ALT), and aspartate aminotransferase

(AST); and an elevated total serum bilirubin.[4,41,42] ALKP is a specific brush border enzyme in the biliary tree, but is also present in other organs, such as the kidneys, intestines, and bones.[43,44] Therefore, an elevation in ALKP may occur with cholestasis, benign disease such as vacuolar hepatopathy, or can be the result of disease affecting organs other than the liver.[43,44] The half-life of ALKP, or the time it takes for ALKP to be reduced to half its original level, is approximately 70 hours, or three days, in normal circumstances.[44] GGT is a liver-specific enzyme that is indicative of biliary disease and cholestasis, and typically rises in conjunction with ALKP.[45]

The aminotransferase enzymes, ALT and AST, are indicative of hepatocellular injury in dogs.[46] ALT, a liver-specific enzyme derived from the cytoplasm of hepatocytes, is considered a more specific and sensitive indicator of hepatocellular injury than AST, which originates from the hepatocyte mitochondria.[45,46] The magnitude of ALT increase is usually greater than that of AST when both are increased due to hepatic injury, in part because of the longer half-life of ALT and the greater proportion of AST that is bound to mitochondria.[46] Hepatic causes of increased serum ALT activity, with or without increased AST activity, include hepatocellular necrosis, injury, or regenerative/reparative activity.[46] Increased serum ALT activity can also be affected by extrahepatic factors.[46] Muscle injury and hemolysis can cause increases in serum transaminase activity, but AST is generally higher than ALT when both are concurrently increased.[46]

Aside from liver enzymes, several other chemistry profile parameters, including serum protein, glucose and blood urea nitrogen (BUN), may be used as indicators of hepatic function or disease.[25] The liver is the exclusive site of albumin synthesis.[25] Because the half-life of albumin is eight to nine days, serum hypoalbuminemia is most often seen in chronic hepatic disorders

such as cirrhosis.[25] The liver also synthesizes many non-immunoglobulins so hepatic disease may also result in a hypoglobulinemia.[25] However, since many non-immunoglobulins are acute-phase reactants whose hepatic production is increased during systemic inflammatory disease, acute liver disease or early chronic liver disease may be accompanied by a hyperglobulinemia.[25] The major function of the liver in carbohydrate metabolism is to maintain a normoglycemia during a fasting state.[25] The liver has a large reserve for maintaining normal serum glucose levels, so that more than 70% of hepatic function must be lost before hypoglycemia occurs. In acute hepatocellular injury, hypoglycemia may be an early indicator of severe hepatic failure.[25] A decreased BUN may be seen in chronic or atrophic liver disease, although a decrease in BUN is not specific for liver disease as it may be influenced by hydration status, dietary protein content, gastrointestinal hemorrhage, or concurrent renal disease.[25] A high-normal serum cholesterol level or hypercholesterolemia are suggestive of posthepatic biliary obstruction.[31]

Fasting and post-prandial bile acids measurements are both sensitive and specific for determining the presence of liver disease or assessing hepatic functionality.[47] Enterohepatic circulation of bile acids is very efficient, with 90-95% of bile acids extracted from the portal circulation on their first pass through the liver.[47] Normally, bile acids in circulation are very low, but the liver loses its ability to remove bile acids from circulation in almost all forms of liver dysfunction.[47] Previous studies have shown 100% specificity for hepatic disease at fasting bile acids greater than 20 umol/L and postprandial bile acids greater than 25 umol/L.[47,48]

Amylase and lipase were elevated in a minority of cases of a GBM when measured, which may indicate reactivity or inflammation within the pancreas, while hypercholesterolemia was elevated in the majority of cases when measured.[4,41,42] Lipases originate from a variety of cells,

including pancreatic, hepatic, and gastric cells.[49] Many or all of the different lipases contribute to the total serum lipase activity measured by traditional assays, resulting in a wide reference interval and low sensitivity and specificity of serum lipase activity for pancreatitis.[49] Although all lipases act to hydrolyze triglycerides, they possess different amino acid sequences because they are encoded by different genes.[49]

In recent years, immunoassays were developed that detect and measure the unique structure of pancreatic lipase, allowing measurement of pancreatic lipase without interference from other lipases.[49] Known as the Specific Canine Lipase Immunoreactivity test, the time required to obtain results was usually 24 hours or more.[49] A rapid semi-quantitative test based on the same methodology but read by visual inspection, known as the SNAP canine Pancreatic Lipase Immunoreactivity test (SNAP cPL), was developed for in-clinic use and allowed faster screening for pancreatitis in acutely ill animals.[49] This test incorporates a reference spot that corresponds to the upper limit of the reference interval for lipase in dogs (200 ug/L) and a sample spot that is visually compared with the reference spot.[49] Results of this rapid assay are interpreted as normal, if the color of the sample spot is less intense than that of the reference spot, or abnormal if the color of the sample spot is equal to or more intense than that of the reference spot.[49] The sensitivity of the SNAP cPL assay for canine pancreatitis has been reported to be very high, ranging from 92-94%, whereas its specificity ranges from 71-78%.[49,50] Therefore, the main use of the test is to eliminate pancreatitis from the differential diagnosis list based on a test result within the reference interval, as the diagnosis of pancreatitis cannot be based on an abnormal SNAP cPL alone.[49]

Since the renal threshold for bilirubin is low, bilirubinuria may be the first indication of bile duct

obstruction in the dog since it commonly precedes icterus.[51] Extrahepatic biliary obstruction shuts down the production of urobilinogens, resulting in conjugated bilirubinuria, without urobilinogens.[9] However, small amounts of bilirubin (trace to 1+) are excreted into the urine in normal dogs.[9,52] Since unbound conjugated bilirubin is filtered and excreted by the kidneys, bilirubinuria is the unbound, nonreabsorbed conjugated bilirubin fraction.[9] Bilirubinuria is seen with intravascular hemolysis because unbound hemoglobin is converted to bilirubin in the kidneys and excreted in the urine.[9] When hemolysis is not present, bilirubinuria is indicative of cholestatic liver disease.[9] Since variables, such as exposure to light, affect the detection of urobilinogen and since normal dipstick methods cannot detect its total absence, the lack of urobilinogen in the urine must be interpreted with caution.[51] Similar to bilirubin, trace amounts of protein in the urine are not considered abnormal.[52] A positive dipstick reading for protein may occur in adequately concentrated urine and still represent normal protein excretion.[52]

Coagulation abnormalities are quite common in dogs with hepatobiliary disease.[25] Hepatocytes synthesize all coagulation factors, except for factor VIII, and are also the site of activation of vitamin K-dependent clotting factors.[25] The liver also functions as an inhibitor of coagulation, fibrinolysis, and fibrinolytic proteins and is responsible for the clearance and catabolism of the by-products of coagulation.[25] Vitamin K deficiency may occur secondary to chronic bile duct obstruction as it interrupts the enterohepatic circulation of bile acids, resulting in intestinal bile acid deficiency and malabsorption of fat-soluble vitamin K.[25] Oral antibiotics may also contribute to a vitamin K deficiency due to the destruction of vitamin K-generating bacteria.[25] Prolongation of the prothrombin time is the first coagulation abnormality seen with vitamin K deficiency because factor VII has the shortest plasma half-life.[25] Despite the possibility of

abnormalities on coagulation tests, spontaneous hemorrhage in animals with hepatic disease is rare.[25] Hemorrhage is more likely to occur with a challenge to hemostasis, such as venipuncture, hepatic biopsy or gastric ulceration.[25] Depletion of more than 70% of any factor must be present to show prolongation of coagulation times.[25] Regardless, coagulation times are recommended prior to any surgery where hepatobiliary disease is suspected or diagnosed.[25,53] If coagulation times are not possible, a mucosal bleeding time to assess for prolonged coagulation or the administration of vitamin K for 24-48 hours prior to surgery is suggested.[53]

Abdominal radiography is rarely helpful in establishing a definitive diagnosis of a GBM.[1] Most often, diffuse hepatomegaly is present, causing a substantial portion of the caudal liver margin to project beyond the costal arch and, most importantly, rounding of the hepatic margins.[54] If substantial hepatomegaly is present, the stomach may be displaced caudodorsally in the lateral view and caudally and toward the left in the ventrodorsal view.[54] The gallbladder itself is rarely visible but, in cases of severe gallbladder distension, a mass-like effect may be present in the right cranioventral abdomen.[1,54]

Abdominal ultrasonography has become the predominant method of diagnosing the presence of a GBM or distinguishing biliary obstruction from hepatocellular disease in clinically icteric animals when serum biochemical findings are ambiguous.[4,7,55,56,57] Ultrasonographic findings of a GBM may include a distended gallbladder with centrally suspended luminal content and a hypoechoic intraluminal rim, intraluminal stellate pattern, echogenic striations or the classic "kiwi fruit sign", a thickened gallbladder wall, or the presence of non-dependent intraluminal contents or sludge.[57] A hypoechoic ring around the gallbladder may indicate wall edema or early rupture, while the presence of free fluid or localized echogenic hepatic parenchyma and

intra-abdominal fat are indicative of bile leakage and peritonitis.[57] Multiple views or movement of the patient from a recumbent to standing position may allow the clinician to determine if non-dependent sludge in the gallbladder lumen is mobile or non-mobile.[58] A positive sonographic Murphy sign, a term used to describe focal pain or discomfort when pressure is applied to a specific area in the abdomen but is generally used to assess for gallbladder pain, may be present.[58]

A recent study attempted to correlate the ultrasonographic appearance of GBM with clinical signs.[4] Symptomatic and asymptomatic dogs were classified into six groups based on the ultrasonographic appearance of the gallbladder contents: immobile echogenic bile (23%), incomplete stellate pattern (30%), typical stellate pattern (12%), kiwi-like pattern and stellate combination (26%), kiwi-like pattern with residual central echogenic bile (9%), and kiwi-like pattern (0%).[4] Although gallbladder rupture was most common with an incomplete stellate pattern, no significant correlations were found between ultrasonographic patterns of GBM and clinical disease status or gallbladder rupture.[4] These findings indicated that ultrasonographic patterns of gallbladder mucoceles may not be a valid basis for treatment recommendations in dogs.[4]

Another study compared the preoperative ultrasonographic appearance of GBM and macroscopic findings for gallbladders and their contents in eleven dogs that underwent cholecystectomy.[55] The dogs were classified into three ultrasonographic patterns: hyperechoic content filling the entire gallbladder and precipitated immobile content (pattern 1), a somewhat thinner hypoechoic area in the exterior layer with a less distinctive border adjacent to the internal hyperechoic area with a moth eaten appearance within the internal hyperechoic

area (pattern 2), and a thick hypoechoic area in the external layer with a distinctive border adjacent to a prominent internal hyperechoic area (pattern 3).[55] The macroscopic findings of the contents mainly consisted of biliary sludge and concentrated bile in pattern 1, a soft mucus mass in pattern 2, and an elastic mucus mass in pattern 3.[55] Chronic cholecystitis was found in all dogs histopathologically examined with hyperplasia of the gallbladder mucosa, increased mucus production, and gallbladder wall necrosis in some cases.[55] No correlation was found between patterns of the GBM and prognosis but content completely filling the gallbladder lumen seemed to correlate with gallbladder dysmotility and increased risk of gallbladder necrosis regardless of content type.[55]

In cases of biliary obstruction (posthepatic hyperbilirubinemia) without a GBM, ultrasound findings depend on the duration and completeness of obstruction.[58] The intrahepatic bile ducts are not visualized in normal dogs, while the extrahepatic biliary ducts are usually poorly visualized in normal dogs owing to overlying bowel gas and, somewhat, to the thin walls of the biliary tree.[58] Under optimal conditions, the proximal common bile duct may be seen to have parallel echogenic walls approximately 0.2 to 0.3 cm apart ventral to the portal vein.[58] The diameter of the common bile duct in normal dogs is reported to be less than 0.3 cm.[58]

Marked gallbladder distension is one of the first ultrasonographic indications of complete obstruction.[58] The cystic duct appears larger and more tortuous than is normally seen in fasted or anorectic animals where the gallbladder may also appear to be distended.[58] Likewise, the common bile duct becomes dilated and tortuous in appearance with variable degrees of dilation, sometimes resulting in a “too many tubes” sign which refers to visualization of the dilated common bile duct and dilated intrahepatic ducts clustered around portal vessels.[58] As

opposed to normal anechoic bile, sediment or mucus may be noted in the common bile duct, which may be referred to as a mucoduct, secondary to biliary stasis.[58] In some cases, ductal dilation may be insufficient for detection of biliary obstruction.[58]

Suspected gallbladder distention may be evaluated by estimating the gallbladder volume using the formula:

Gallbladder volume = length x width x height x 0.53.[5]

A normal gallbladder volume after an eight to 12 hour fast is less than 1 ml/kg of body weight, while a gallbladder volume greater than 1 ml/kg of body weight indicates increased volume and possible reduced contractility.[5]

Acute pancreatic disease or pancreatitis is usually diagnosed on ultrasound by recognizing an enlarged pancreas or an ill-defined hypoechoic mass effect surrounded by hyperechoic peripancreatic fat in the pancreatic region.[59,60] In chronic pancreatitis, the pancreas may become thickened, hyperechoic, or be of mixed echogenicity.[60] Local pressure applied to the right cranial abdomen may result in a positive sonographic Murphy sign.[59] Variable amounts of free fluid, focal peritonitis, fat saponification from the release of pancreatic enzymes, and duodenal and stomach wall thickening with functional ileus may be present in more severe acute or subacute pancreatitis.[59] Signs of biliary obstruction with bile duct dilation, gallbladder enlargement, and elevation of serum bilirubin may be present in acute stages of pancreatitis where the duodenal papilla is involved or obstructed.[59] It should be noted that pancreatitis cannot be differentiated from pancreatic neoplasia, which may have ultrasonographic characteristics similar to acute pancreatitis, or focal septic peritonitis solely on the basis of the

ultrasound appearance.[59] However, pancreatic tumors are rare, which favors the diagnosis of pancreatitis when an abnormal pancreas is present.[59]

As with pancreatitis, inflammatory or neoplastic lesions in the upper duodenum may result in obstruction of the duodenal papilla.[61,62] On ultrasound, inflammatory lesions tend to maintain visible wall layer integrity with focal or diffuse mural wall thickening, whereas a complete loss of normal wall layering detail and transmural wall thickening is typically, but not always, seen with intestinal neoplasia.[61]

Acute cholecystitis may have a variety of sonographic appearances but gallbladder wall thickening is usually present.[58] A positive Murphy sign may be present.[58] Emphysematous cholecystitis, a form of acute cholecystitis, results in gallbladder wall thickening as well as reverberation artifact secondary to gas-forming organisms in the lumen.[58] Necrotizing cholecystitis, another form of acute cholecystitis and seen with GBM, is characterized by marked wall irregularity or asymmetric wall thickening, usually with pericholecystic fluid accumulation secondary to necrosis of the gallbladder wall.[58] Chronic cholecystitis is also characterized by a thickened to echogenic gallbladder wall but usually presents in a less acute form than acute cholecystitis.[58] In severe cases of chronic cholecystitis, inflammation and fibrosis of the gallbladder wall may prevent normal distension of the gallbladder, making the gallbladder difficult to locate on ultrasound.[58]

The frequency of canine cholelithiasis is low.[63] Choleliths are generally subclinical and are often discovered as incidental findings within the gallbladder as dense structures with an echogenic interface and distal acoustic shadowing during ultrasonography.[58,63] However, signs similar to a

GBM, such as anorexia, vomiting, diarrhea, lethargy, icterus, and abdominal pain may be present, while severe disease manifests when choleliths cause extrahepatic biliary tract obstruction, or rupture of the gall bladder or common bile duct.[63] Cholelith movement or impaction within the cystic duct, common bile duct, or at the duodenal papilla, causes intense local pain or referred pain which may be localized to the right upper abdominal quadrant, to the epigastrium, or to the retrosternal region.[5] Calculi in the common bile duct may be difficult to detect because of interference from bowel gas.[58]

Hepatobiliary neoplasms that may be associated with extrahepatic bile duct obstruction include biliary adenoma / adenocarcinoma, biliary cystadenoma, hepatocellular carcinoma, and lymphosarcoma.[5,64] Pancreatic adenocarcinoma and alimentary neoplasia such as adenocarcinoma, lymphoma, leiomyoma or leiomyosarcoma, may also occur.[5,64] Ultrasound is important for localizing a mass in relation to the gallbladder and common bile duct, and determining the resectability of the mass to alleviate biliary obstruction.[64]

Medical management of GBM may be appropriate for asymptomatic or mildly affected patients with no indication of gallbladder rupture, evidence of perigallbladder inflammation, or significant elevations of the white blood cell count or liver enzymes.[1,57] Owners should be advised to carefully monitor their pets for clinical signs associated with progression of the disease, and be aware that patients treated medically for a GBM may acutely decompensate because of gallbladder rupture, extrahepatic biliary obstruction, bile peritonitis, or sepsis.[1,57]

Hepatoprotectants have been promoted for their potential role in the ancillary treatment of hepatobiliary disease in dogs.[65] These products include both prescription drugs and nondrug

dietary supplements.[65] Ursodeoxycholic acid is a choleretic and hepatoprotectant medication that promotes the secretion of thinner bile by reducing cholesterol saturation in the bile; it reduces the hepatocyte toxic effects of bile salts, and may protect hepatic cells from toxic bile acids in cases of cholestasis.[66] Ursodeoxycholic acid also reduces hepatocellular inflammatory changes and fibrosis in cases of hepatitis.[66] The use of ursodeoxycholic acid in cases of biliary obstruction, as with a GBM, is controversial. Some have argued that biliary obstruction must be ruled out to warrant its use, while others state that ursodeoxycholic acid does not have prokinetic effects on gallbladder motility and, therefore, is not contraindicated if biliary obstruction is present.[1,66]

S-adenosylmethionine (SAMe) is produced endogenously within the body and is an essential part of major biochemical pathways within the liver.[67] Additionally, SAMe is a precursor of glutathione, an important component of metabolic processes and cell detoxification within the liver.[67] Normally, the liver produces ample amounts of SAMe; however, in liver disease or the presence of hepatotoxic substances, SAMe and, thus, glutathione may be deficient.[67] Supplementation of synthetic SAMe, an antioxidant nutraceutical, may be chosen as an adjunctive treatment in cases of liver disease although its efficacy is questionable.[65,67]

Other nutraceuticals that are commonly used in veterinary medicine to treat hepatobiliary disease include silymarin, vitamin E, and N-acetylcysteine.[65] Derived from the milk thistle plant, silymarin is thought to exert antioxidant, anti-inflammatory, and anti-fibrotic effects.[65,68] In a recent study, denamarin, a commercially available product that contains both SAMe and silymarin, was shown to reduce the hepatotoxic effects of the chemotherapeutic agent chloroethylcyclohexylnitrosourea.[69] The primary physiologic role of vitamin E is as an

antioxidant.[65] Vitamin E supplements have been recommended for dermatologic and hepatobiliary diseases (cholestatic and necroinflammatory hepatopathies) in which antioxidant activity may be of benefit.[65,68] N-acetylcysteine is a formulation of an amino acid that has traditionally been used as an acetaminophen antidote in veterinary medicine.[65] N-acetylcysteine may be used to replenish intracellular cysteine and glutathione levels, which are important for overall hepatic health.[65,68] Several other potentially hepatoprotective effects have been reported, including an effect on vascular tone that may improve oxygen delivery in acute liver failure, effects on hepatic mitochondrial energy metabolism, and potential effects on inflammation.[65,70]

A histamine H2-receptor antagonist, such as famotidine, or a proton pump inhibitor, such as omeprazole, reduces gastrointestinal acid production and increases gastric pH if gastroenteritis secondary to a GBM is suspected.[71,72] Proton pump inhibitors have been shown to be superior to H2-receptor antagonists in increasing gastric pH.[73] Antiemetics, such as maropitant, and a fat restricted diet are commonly used to control nausea or vomiting and hyperlipidemia, respectively.[1] Maropitant mimics the structure of substance P, a key neurotransmitter in the stimulation of vomiting, and binds to neurokinin 1 (NK-1) receptors so they cannot bind substance P, thus decreasing stimulation of the emetic center.[74] Maropitant has also been shown to reduce the mean alveolar concentration of gas anesthetics through the inhibition of visceral pain.[75] NK-1 receptors and substance P have been reported in pain pathways at the level of the central nervous system and peripheral nervous system, as well as visceral tissues such as the bladder, esophagus and colon.[75] By blocking the binding of substance P to NK-1 receptors in the nervous system, visceral pain is reduced.[75] Additionally, maropitant has been

shown to reduce adverse side effects, such as vomiting, commonly seen with the administration of opioid medications.[76]

Oral or injectable analgesics may be elected if the patient is painful. Different classes of oral analgesics, including opioids or opioid-like medications, N-Methyl-D-aspartate (NMDA) receptor antagonists, NSAIDs, or combinations of these medications, are effective at controlling both postoperative and chronic pain.[77] NSAIDs should be used with caution in patients with preexisting gastrointestinal, renal, or hepatic disease as they are metabolized by the liver and may cause hepatotoxicity, renotoxicity, or gastrointestinal ulceration.[78] Commonly used injectable mu-receptor agonist opioids, including morphine, hydromorphone, and oxymorphone, are metabolized by the liver, primarily by glucuronidation, so caution must also be used when administering these medications to patients with liver disease.[79,80,81]

The use of antibiotics in the medical or surgical management of GBM is generally recommended in dogs undergoing gallbladder surgery, particularly when there is a suspicion or confirmation of gallbladder rupture.[7,41,82] Previous studies analyzing bacterial culture results at the time of gallbladder removal because of a GBM yielded controversial results with positive cultures as high as 66.7% to as low as 9.1%, with an average rate of positive bacterial colonization of approximately 13.4%.[41,42,55,56,82,83] In other surgically treated biliary diseases, higher rates of positive culture results have been reported, reaching 35-50% in some cases.[7,41] The variability in positive culture results is thought to be secondary to the use of intraoperative or perioperative antibiotics which may play a role in decreasing the number of positive culture results.[41,82] Ultrasound-guided cholecystocentesis to collect a bile sample for bacterial culture may be considered in animals where medical management of a GBM is pursued.[11] However,

this procedure should be performed with caution as complications, such as bile leakage, bradycardia due to vagal stimulation, bacteremia, and local hemorrhage are possible.[5,11] Antibiotics that are commonly used in cases of biliary disease, particularly in cases of a GBM, include the following: ampicillin or amoxicillin (20 mg/kg q8-12h) due to their effectiveness against anaerobes and gram-negative aerobes; enrofloxacin (5-10 mg/kg q12-24h) due to its activity against aerobes and gram-negative/gram-positive cocci and bacilli; metronidazole (10-15 mg/kg q12h) due to its effectiveness against anaerobes; and cefazolin (10-30mg/kg q8h) due to its effectiveness against gram-positive anaerobic bacteria.[1,53,84] Long-term therapy with metronidazole may result in neurotoxicosis or hepatotoxicosis.[84] Since metronidazole is primarily metabolized in the liver, reduction of the dose to 7.5-10 mg/kg should be considered in patients with hepatic disease.[84]

Two cases of nonsurgical resolution of a GBM in dogs are reported in the literature.[85] The first dog had a history of signs of gastrointestinal tract disease, including inappetence, vomiting and diarrhea, hypercholesterolemia, and high serum liver enzyme activity.[85] A GBM and hypothyroidism were diagnosed through abdominal ultrasound and thyroid hormone and thyroid-stimulating hormone levels, respectively.[85] The dog was treated with SAMe, omega-3 fatty acids, famotidine, ursodeoxycholic acid, and levothyroxine, a synthetic thyroid hormone.[85] Complete resolution of the GBM on ultrasound was evident within three months of treatment.[85] The second dog had a history of chronic, intermittent diarrhea, recurrent otitis and hypercholesterolemia.[85] It, too, was diagnosed with a GBM through ultrasonography and hypothyroidism by means of decreased serum thyroid hormone and thyroid-stimulating hormone levels.[85] Treatment consisted of fenbendazole, ursodeoxycholic acid, levothyroxine

and a hypoallergenic diet.[85] Ultrasonography revealed that the GBM was resolving one month after treatment was started with complete resolution of the GBM within four months of treatment.[85] Although it is impossible to determine whether correction of the hypothyroidism or other medical management had any effect on resolution of the GBM in these dogs, these cases suggest that, although rare, treatment of an underlying endocrinopathy combined with appropriate medical management and regular examinations may eliminate the need for surgery in some cases of GBM.[85]

Cholecystectomy is the recommended treatment for dogs diagnosed with a GBM for several reasons. The documented histological evidence of mucosal hyperplasia and cholecystitis in GBM indicates that the gallbladder is diseased.[15] Also, the congealed bile and mucus along with gallbladder distension characteristic of a GBM is unlikely to pass with the use of choleretics.[15] Lastly, the risk of gallbladder rupture, bile peritonitis, and secondary bacterial infections is high with GBM until the gallbladder is removed.[15] Bile peritonitis is common, with overt gallbladder rupture reported to be as high as 40-60% of surgical cases.[1] The surgical procedure generally involves ligating the cystic duct and artery prior to removal of the gallbladder.[53] However, prior to removing the gallbladder, patency of the common bile duct must be ensured. In appropriate cases, the patency of the common bile duct may be determined by manual expression.[53] Techniques involving flushing of the common bile duct either through normograde catheterization by cholecystotomy prior to performing the cholecystectomy, or retrograde via the major duodenal papilla after duodenotomy have been described.[41,53]

Cholecystotomy to remove the debris from the gallbladder lumen is not recommended due to potential microscopic wall necrosis, which may lead to post-operative gallbladder rupture, and

reported recurrence of GBM formation in dogs where cholecystotomy was initially performed.[5] Submission of the resected gallbladder, along with a liver biopsy obtained during the surgery, for histopathology is warranted in all cases of GBM.[1] The most common liver diagnoses include cholangiohepatitis, biliary hyperplasia, and cholestasis, as well as portal fibrosis and hepatitis.[41] Since biliary excretion is the major elimination route of copper, increased hepatic copper accumulations secondary to cholestasis may be seen.[37] Perioperative pancreatitis is common but is not associated with an increased risk of perioperative death.[1]

Stabilization and correction of dehydration or electrolyte abnormalities, in addition to the assessment of coagulation profiles, are important prior to surgery.[53] The fluid rate is determined by calculating metabolic fluid requirements while estimating dehydration or monitoring ongoing fluid losses.[86] The calculation of resting energy expenditure:

$$\text{REE} = \text{ml water} = (30 \times \text{body weight (kg)}) + 70,$$

where metabolism of 1 kcal of energy equals 1 ml of water consumed, allows the determination of the daily water requirements of a patient in a 24-hour period, although other formulas may be used.[86] Replacing fluid loss secondary to dehydration, hypovolemia, or ongoing losses (vomiting, diarrhea, or renal loss) may be calculated using the formula:

$$\text{Dehydration (\%)} \times \text{body weight in kg} \times 1000 = \text{ml fluid deficit.}[86,87]$$

This deficit is generally replaced over a six to 24-hour period, depending on a patient's stability and ability to handle the volume administered.[86] A subcutaneous (SQ) route of fluid administration is best used to prevent losses and is not adequate for replacement therapy in any case except for very mild dehydration.[87] Anesthetic fluid rates generally provide the

maintenance rate plus any necessary replacement rate at less than 10 ml/kg/hr but may be adjusted based on patient assessment.[87] Propofol, a short acting hypnotic agent, is recommended for induction of anesthesia in patients with underlying hepatic disease.[88,89] It is rapidly metabolized by the liver via glucuronide conjugation in healthy dogs, so a prolonged anesthetic recovery may be expected with its use in dogs with liver disease.[88,89] Benzodiazepines, often combined with an opioid, may also be considered for anesthetic premedication in animals with hepatic disease, although lower doses are recommended.[88] Isoflurane or sevoflurane are recommended for anesthetic maintenance.[88]

With a perioperative mortality rate ranging from approximately 20-40%, the overall prognosis for dogs with a GBM undergoing cholecystectomy is guarded.[41] Postoperative complications include bile peritonitis, pulmonary thromboembolism, pneumonia, pancreatitis, sepsis, surgical dehiscence, disseminated intravascular coagulation, and cardiac arrest.[1] However, if the dog survives the initial two week post-operative period, long term survival is excellent.[41] Long term survival rates for dogs surviving surgery and the immediate post-operative period has been reported to be as high as 66%.[83] Negative prognostic factors include an older age, a higher degree of liver enzyme elevation, a higher degree of white blood cell count elevation, a post-operative elevation of serum lactate concentrations, and post-operative hypotension.[41,55] In one study, the mean age of death cases was 11.8 years compared to 8.4 years of age for surviving cases.[55] Although no clear association between liver enzyme elevations, total bilirubin elevation, appearance of the gallbladder and prognosis was found, the mean WBC count was approximately 2.5 times higher in death cases compared to surviving cases.[55] A significantly elevated WBC count in combination with significant liver enzyme elevation may possibly reflect

disease severity, degree of tissue damage, biliary peritonitis or gallbladder rupture.[55,82]

Clinical Report

A nine-year-old spayed female Yorkshire Terrier-Miniature Schnauzer mixed breed dog presented for lethargy, decreased appetite and one episode of vomiting after the dog ingested a potato cake the previous day. The dog became lethargic later in the day and only ate a small amount of her normal diet for dinner; she did not eat breakfast prior to presentation at the clinic. Gagging, coughing, dyspnea, or diarrhea were not reported by the owner. The dog had received all pertinent vaccinations seven months prior to the onset of clinical signs. Previous medical history included bilateral yeast otitis externa. The owner fed a well-balanced, commercially available diet. No previous treatment for lethargy, decreased appetite, or vomiting was noted in the medical record.

On physical examination, the dog was bright, alert and responsive. Her mucous membranes were slightly tacky with a normal capillary refill time of two seconds. The skin turgor along the dorsum was mildly decreased. Oral examination showed minimal to no periodontal disease or dental calculus. No ptyalism or evidence of oral pain was present within the mouth or when the mouth was fully opened. There were no orolaryngeal masses or foreign bodies. The masticatory musculature was of normal firmness and size on palpation. Examination of the eyes revealed clear corneas, normal anterior chambers and lenses, white sclera and pink conjunctiva with no signs of exophthalmos or enophthalmos. Mild to moderate pressure applied to each eye was negative for pain. Examination of the nostrils revealed clear nasal openings with no signs of discharge, crusts or inspiratory stridor. The external ear canals were pink with minimal

ceruminous debris. Palpation of the peripheral lymph nodes was normal with no evidence of lymphadenopathy. Thoracic auscultation revealed a heart rate of 150 beats per minute (110-120 beats per minute) with a normal rhythm.[90] No cardiac murmur was detected. Femoral pulse quality was strong and without pulse deficits. The respiration rate was 45 breaths per minute (15-30 breaths per minute).[90] Thoracic auscultation revealed normal inspiratory and expiratory movement of air in the lungs with no auditory crackles or wheezes present. No coughing was elicited on mild tracheal palpation. On abdominal palpation, the abdomen was mildly tense with no obvious pain or palpable abnormalities. Several cyst-like growths were noted along the caudal dorsum. Cranial and peripheral neurologic function was normal with no ataxia or proprioceptive deficits. No lameness was noted when walking. The rectal temperature was 39.1°C (37.5-39.2°C).[90] The dog's body weight was 5.2 kg and body condition score was 5/9.[91]

Based on the history and physical examination, the initial problem list included the following: anorexia, vomiting, mild abdominal discomfort due to tenseness during palpation, mild tachycardia and tachypnea, and an estimated 5-7% dehydration due to slightly tacky mucous membranes, a mild decrease in skin turgor, and a mildly elevated heart rate.[86] Many of the causes of anorexia, vomiting, and abdominal discomfort are similar: disorders of the gastrointestinal tract such as inflammatory disease, intestinal foreign bodies and obstruction; intussusception; parasitic or viral infections; bacterial disease or overgrowth including Helicobacter spp., ulceration, obstipation or neoplasia; and abdominal disorders such as pancreatitis, peritonitis, hepatobiliary disease, or non-gastrointestinal neoplasia.[92,93] Other causes of vomiting or anorexia without abdominal pain or discomfort include metabolic or

endocrine disorders such as anemia, hypoadrenocorticism, diabetes mellitus, hepatic disease, electrolyte or acid base disorders, intoxicant ingestion, or dietary intolerance.[92,93] Anorexia, by itself, may result from many diseases that affect the ability to smell food or masticate, such as nasal disease, skull or mandibular trauma, temporo-mandibular joint disease, severe dental disease, masticatory myositis, retrobulbar masses or abscesses, or neurologic abnormalities as well as thoracopulmonary illnesses including pneumonia, pleural effusion, or airway disease.[92]

Anxiety, pain, metabolic disease, cardiovascular or pulmonary disease, endocrine disorders and hypertension may cause tachycardia and tachypnea.[94] Possible causes for mild dehydration include decreased fluid intake as a result of the many causes of anorexia, normal fluid loss secondary to typical urine output and evaporation from the lungs, and increased fluid loss from vomiting or diarrhea.[95] Differential diagnoses for the cyst-like growths on the caudal dorsum include sebaceous gland cysts, follicular cysts, or epidermal cysts but were not considered relevant to the presenting complaint.[96]

The diagnostic plan included abdominal radiographs and collection of blood and urine for a complete blood count, chemistry panel, in-house SNAP canine Pancreatic Lipase Immunoreactivity Test[a] (SNAP cPL), fecal analysis and urinalysis. The owner declined the majority of the initial plan, opting only for the SNAP cPL test (Table 1) as the sole diagnostic test. Conservative treatment for suspected gastroenteritis secondary to dietary indiscretion was elected. A balanced electrolyte solution[b] 30 ml/kg (150 ml) SQ and famotidine[c] 0.5 mg/kg (2.6 mg) SQ were administered in the craniodorsal back and right lateral hind limb, respectively. Maropitant[d] 2.3 mg/kg (12 mg) PO q24h was prescribed. Four cans of a bland diet[e], which contained 23 g/Mcal of fat, were also prescribed with instructions to feed the bland diet

Table 1: SNAP Canine Pancreatic Lipase Immunoreactivity Test, Day 1 (**Bold** indicates outside of reference range)

Test	Result	Reference Range
SNAP cPL	Normal Lipase level	Normal Lipase level = sample spot is lighter in intensity than the reference spot Abnormal Lipase level = sample spot equal to or darker than the reference spot

exclusively over the next 24 -48 hours then gradually mix the bland diet in with the normal diet if the pet's appetite was normal.[24] The dog ate a small amount of bland diet when it was offered in the examination room. The owners were instructed to have the dog rechecked if vomiting or inappetence continued, or if any other abnormalities were seen.

The dog presented again three days later. The owner reported that the dog had become increasingly lethargic or inactive over the previous 24 hours. Her appetite was still decreased although she was eating small amounts of the bland diet. Her water intake was decreased, and the urine was dark in color. The owner reported no further vomiting and no diarrhea.

On physical examination, the dog was quiet, alert, and responsive. She walked around the exam room without evidence of lameness or posture manipulation. Her mucous membranes remained slightly tacky with a capillary refill time of two seconds. Decreased skin turgor was still present. Mild icterus was noted in the mucous membranes, scleras, inner pinnae and ventral abdomen. The heart rate was 140 beats per minute (110-120 beats per minute) and the respiration rate was 50 breaths per minute (15-30 breaths per minute).[90] On abdominal palpation, the abdomen was tense with moderate discomfort or pain in the cranial abdomen but palpable structural abnormalities were not evident. Using a numerical pain rating scale, the pain was assessed to be four out of ten.[40] Palpation of the thoracolumbar back did not reveal obvious or localized pain. Proprioception in the hind limbs was normal and there was no visible evidence of trauma around the cranial abdomen. Femoral pulse quality was normal and synchronous. Oscillometric mean arterial pressure[f] (MAP) obtained from the right forelimb in a sternal position was normal at 110 mmHg (85-120 mmHg).[97] Her rectal temperature was 39.2^{o}C (37.5^{o}C-39.2^{o}C).[90] Her body weight was 5.09 kg, indicating mild weight loss from the previous

examination, and her body condition score was 4.5/9.[91]

A revised problem list included new abnormalities. Lethargy, definitive cranial abdominal discomfort or pain, icterus, decreased water intake and mild weight loss had developed. Similar signs observed during the initial examination, which included a decrease in appetite or anorexia, mild tachycardia, mild tachypnea, and an estimated 5-7% dehydration, were still present.[86] Numerous abnormalities, such as diseases of the cardiovascular, endocrine, neuromuscular and respiratory systems, metabolic disease, electrolyte or acid-base disorders, autoimmune or infectious pathologies, inflammatory conditions, or pain, may result in decreased activity or lethargy.[98] Possible causes of mild to moderate visceral pain localized to the cranial abdomen include hepatobiliary disease, gastrointestinal distension or obstruction, pancreatitis, peritoneal disease and neoplasia.[40]

The primary causes of icterus included conditions that lead to increased biliary production or impaired biliary excretion.[31] The main mechanism of increased prehepatic biliary production is hemolysis, specifically autoimmune hemolysis, although other potential causes of hemolysis include infectious or parasitic disease, zinc toxicosis, or paraneoplastic syndrome.[31,34] Impaired biliary excretion, or cholestasis, generally results from hepatic parenchyma diseases or posthepatic, partial or complete biliary obstruction.[31,34] Examples of hepatic parenchyma disease include acute to chronic hepatitis, infectious disease such as leptospirosis, cirrhosis, hypoxia, toxicosis or drug ingestion, congenital and acquired portosystemic shunting, portal vein hypoplasia, and benign or malignant neoplasms such as lymphoma, hepatic adenoma or adenocarcinoma, mast cell disease or other miscellaneous neoplasia.[31,34] Causes of partial or complete biliary obstruction include inflammatory conditions of the biliary system such as

cholangitis or cholecystitis, GBM, extrahepatic bile duct obstruction via choleliths in the gallbladder or common bile duct, accumulated mucus in the common bile duct or in the duodenal papilla, neoplasia, or duodenal disease and obstruction of the duodenal papilla.[31,34]

Decreased water intake and weight loss were attributed to the same differential diagnoses that may cause anorexia.[92,99] Anxiety, pain, metabolic disease, cardiovascular or pulmonary disease, endocrine disorders and hypertension were still considered the primary differential diagnoses for tachycardia and tachypnea.[94] Possible causes of the estimated 5-7% dehydration include decreased water intake secondary to anorexia and normal fluid loss secondary to typical urine output and evaporation from the lungs.[86,95] Increased fluid loss through vomiting or diarrhea was considered less likely.[95]

The basic diagnostic plan remained unchanged from the initial examination. Venous blood samples were collected for an in-house complete blood count[g] (Table 2), a manual PCV with differentiation (Table 3), and chemistry panel[h] (Table 4). A free catch sample of urine was collected for an in-house urinalysis, including a dipstick reagent strip[i] as well as an in-house urine sediment (Table 5). A fecal sample was obtained by using a fecal loop[j] for an in-house fecal analysis[k] following centrifugation (Table 6). The urine was yellow-orange in color, consistent with the owner's report of discolored urine. The serum was observed to be moderately yellow in color once it was separated from the blood cells. The manual PCV percentage was within the low reference range and was compatible with the automated hematocrit. No evidence of blood parasites, spherocytes, or other red blood cell abnormalities were seen.

Table 2: Complete Blood Count, Day 1 (**Bold** indicates outside reference range)

CBC	Result	Reference Range	Units
Hematocrit	37.1	37.0-55.0	%
Hemoglobin	12.6	12.0-18.0	g/dL
RBC	5.6	5.5-8.5	10^6/uL
MCHC	34.5	32.0-38.5	g/dL
MCV	67.9	60.0-72.0	fL
MCH	23.4	19.5-25.5	pg
RDW	17.5	12.0-17.5	%
RDWa	44.6	35.0-53.0	fL
WBC	12.9	6.0-17.0	10^3/uL
Grans	8.3	3.5-12.0	10^3/uL
% Grans	64.2	0.0-99.9	%
Lymphs	2.6	1.2-5.0	10^3/uL
% Lymphs	20.4	0.0-99.9	%
Monos	**2.0**	0.3-1.5	10^3/uL
% Monos	15.4	0.0-99.9	%
Plt	368	200-500	10^3/uL
MPV	8.4	5.5-10.5	fL

Table 3: Packed Cell Volume (PCV) and Manual Differential, Day 1 (**Bold** indicates outside of reference range)

Test	Result	Reference Range[100]	Units
PCV	38	33-58.7	%
Manual Diff			
Neutrophils	64		%
Bands	0		%
Lymphocytes	22		%
Monocytes	13		%
Eosinophils	1		%
Basophils	0		%
Platelet (est)	Adequate		
Blood parasites	None seen		

Table 4: Chemistry Profile, Day 1 (**Bold** indicates outside reference range)

Chemistry Profile	Result	Reference Range	Units
Albumin	3.0	2.5-4.0	g/dL
ALKP	**>993**	0-140	U/L
ALT	**>1000**	0-120	U/L
AST	**78**	0-60	U/L
AMYL	499	100-1500	U/L
LIPA	39	0-225	U/L
BUN	12	9.0-29.0	mg/dL
Ca	9.8	9.0-12.2	mg/dL
Chloride	**101**	102-120	mEq/L
CHOL	**428**	92-324	mg/dL
CREA	0.5	0.4-1.4	mg/dL
GGT	**57**	0-14	U/L
Glucose	124	75-125	mg/dL
Mg	1.5	1.5-2.4	mEq/dL
PHOS	4.9	1.9-5.0	mg/dL
Potassium	3.8	3.6-5.5	mEq/L
Sodium	150	141-152	mEq/L
Chloride	105	102-120	mEq/L
TBIL	**5.6**	0.0-0.5	mg/dL
Total Protein	6.4	5.5-7.6	g/dL
TRIG	129	30-130	mg/dL
Globulin	3.4	1.6-3.6	g/dL
Na/K ratio	39.5		Ratio

Table 5: Urinalysis, Day 1 **(Bold** indicates out of reference range)

Urinalysis	Result	Reference Range[101]
pH	7.5	6.0-7.5
Protein	**Trace**	Negative
Glucose	Negative	Negative
Ketones	Negative	Negative
Blood	Negative	Negative
Bilirubin	**Large (3+)**	Negative to 1+
Urobilinogen	Normal	Negative
Leukocytes	Negative	0-3
Nitrites	Negative	Negative
SG	1.032	1.015-1.050
Bacteria	None seen	None
Epi Cell	None seen	0-3
Mucus	Negative	Negative
Casts	None seen	None
Crystals	1+ bilirubin	None
Urobilinogen	Negative	Negative

Table 6: Fecal analysis following centrifugation, Day 1

Test	Result	Reference Range
Fecal analysis	Parasite ova were not present in the feces.	Negative test = parasite ova are not present in the feces. Positive test = parasite ova are present in the feces.

Once the results of these tests were completed, a refined problem list indicated a mildmonocytosis, moderate hyperbilirubinemia (TBIL), significant elevations of liver enzymes including ALKP, GGT, ALT, and AST, a mild hypochloremia, a mild to moderate hypercholesterolemia, significant hyperbilirubinuria with mild bilirubin crystaluria, and a trace proteinuria.

Differential diagnoses for the mild monocytosis include mild chronic inflammation or necrosis of various origins.[102] The degree of TBIL elevation on the chemistry panel was consistent with the findings of icterus on the physical examination and bilirubinuria on the urinalysis.[31] Potential causes of the elevated TBIL include previously mentioned hepatic and posthepatic causes as hemolysis did not appear to be present.[31] Since GGT is a liver specific enzyme that is indicative of biliary disease and cholestasis, and typically rises in conjunction with ALKP, potential causes of the elevations of these enzymes were considered together and could be categorized into primary hepatic cholestasis and posthepatic cholestasis.[31,34] Diseases that may cause primary hepatic cholestasis include vacuolar hepatopathy, reactive hepatopathy secondary to other systemic diseases such as hyperadrenocorticism, chronic hepatitis, pancreatitis or gastrointestinal disorders, infectious disease, toxicosis, nodular hyperplasia, passive hepatic congestion as with right side heart disease, hyperlipidemia, or neoplasia.[34]

Posthepatic cholestasis may result from gallbladder diseases such as significant amounts of non-organized biliary sludge, GBM formation, or cholelithiasis, extra hepatic common bile duct obstruction, pancreatitis, and neoplasia.[31,34] Primary bone disease and kidney disease may also cause elevations in ALKP but this was considered unlikely.[34] Significant hepatocellular damage was represented by the elevations of both ALT and AST.[45,46] Considered together, potential

conditions that may lead to elevations of both enzymes include acute or chronic hepatitis, reactive hepatopathy, infectious disease including bacterial, fungal or parasitic disease, cirrhosis, endocrinopathies such as hyperadrenocorticism, toxicosis, copper storage disease, pancreatitis, or neoplasia.[45,46] Other causes of an AST elevation, such as muscle disease or hemolysis, were considered less likely.[45] Possible causes of the mild hypochloremia include decreased chloride intake, chloride loss through vomiting or renal disease, and gastrointestinal stasis or obstruction.[103] Abnormalities that may cause elevated serum cholesterol levels include cholestasis, pancreatitis, endocrinopathies such as hyperadrenocorticism, hepatic disease or insufficiency, and idiopathic hypercholesterolemia.[104] Diabetes mellitus and nephrotic syndrome, additional causes of hypercholesterolemia, were considered less likely since hyperglycemia or azotemia, respectively, were not present.[104] Since the dog was a Yorkshire Terrier-Miniature Schnauzer mix breed and dyslipidemias have been documented in Miniature Schnauzers, breed-associated hypercholesterolemia was also considered.[3]

Although low levels of bilirubinuria may be seen in normal dogs, the main differential diagnoses for the presence of an elevated bilirubinuria, in light of the mild icterus noted on physical examination, include cholestasis secondary to previously mentioned hepatic or posthepatic diseases and hemolysis.[31,34,52] The trace proteinuria was not considered to be clinically significant.[52]

The collected blood within the tube containing ethylenediaminetetraacetic acid was observed for evidence of clotting. A drop of blood from tube was placed on a slide and macroscopically evaluated for red blood cell clumping. Neither clotting within the tube nor signs of visible clumping in the drop of blood were noted. A saline agglutination test (Table 7) was performed

Table 7: Saline Agglutination Test, Day 1

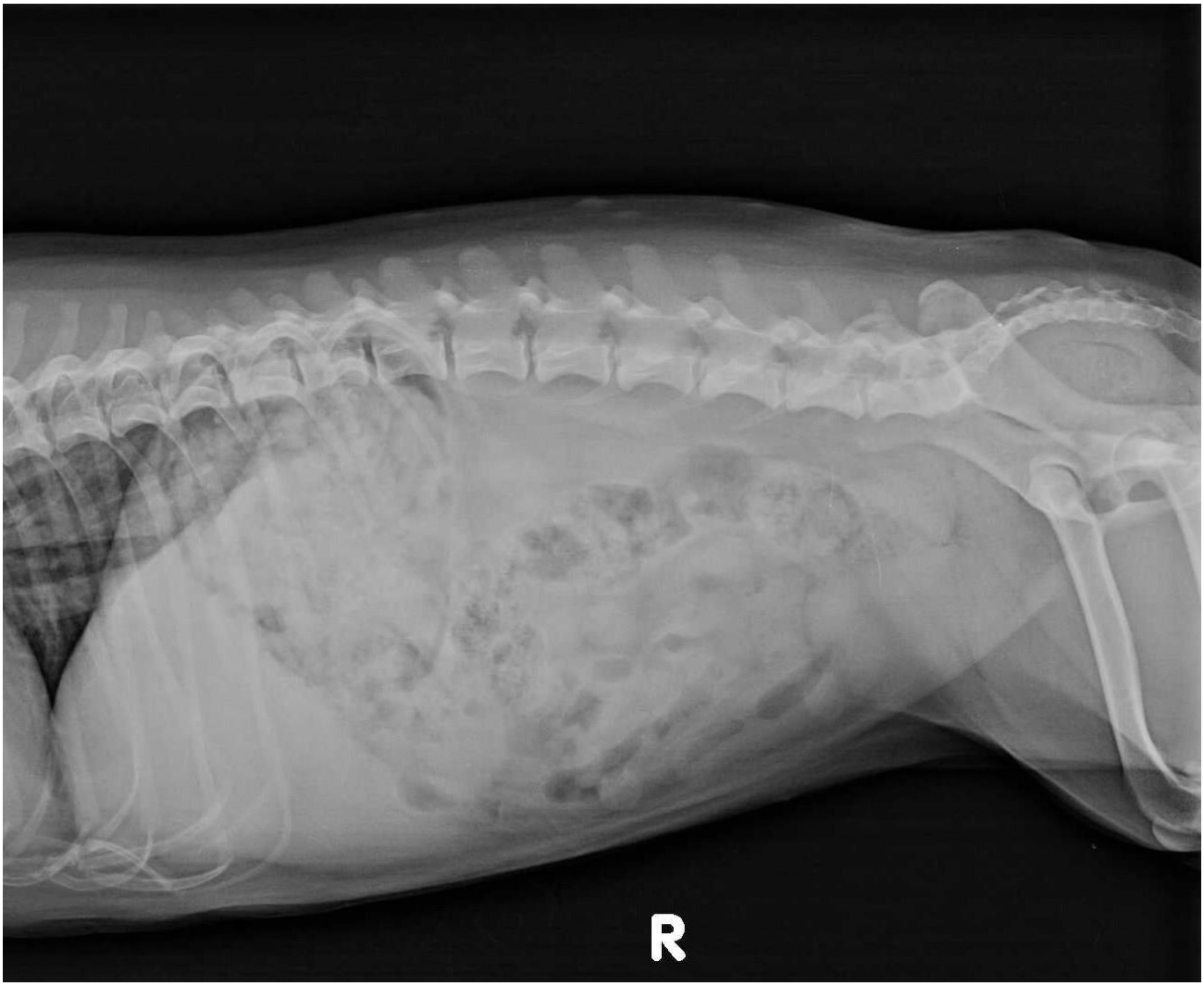
Test	Result	Reference Range
Saline Agglutination Test	Macroscopic and microscopic dispersement of red blood cell clumps.	Dispersement of red blood cell clumping or continued clumping of red blood cells

on the drop of blood.[32] Under the microscope, intermittent to mild rouleaux formation remained but significant rouleaux formation or clumping of the red blood cells was not seen.

Abdominal radiographs[l] (Figures 1 and 2) revealed moderate generalized hepatomegaly on the lateral view. This was indicated by the caudolateral edge of the liver extending beyond the costal margin.[54] Additionally, the enlarged liver appeared to displace the pylorus caudally and dorsally in the lateral view, resulting in a shift of the gastric axis.[54] The caudal margins of the liver appeared to be smooth and rounded, a sign of diffuse hepatomegaly.[54] The pylorus was not obviously visible in the ventrodorsal view but shifting of the pylorus medially, or to the left of its normal position, was suspected, another indication of hepatic enlargement.[54] Loss of detail was noted in the right cranial abdominal quadrant in the ventrodorsal view. The gallbladder was not visible but there was no evidence of choleliths in the region of the gallbladder or common bile duct. The stomach was moderately distended with normal ingesta. The intestines appeared within normal limits with no evidence of ileus, obstruction or gastrointestinal foreign body. The spleen, kidneys, and urinary bladder were normal in size and appearance. The visible thoracic and lumbar vertebrae as well as the pelvis were also normal in appearance. An unstructured interstitial pattern was noted in the visible portions of the caudal lung lobes.[105]

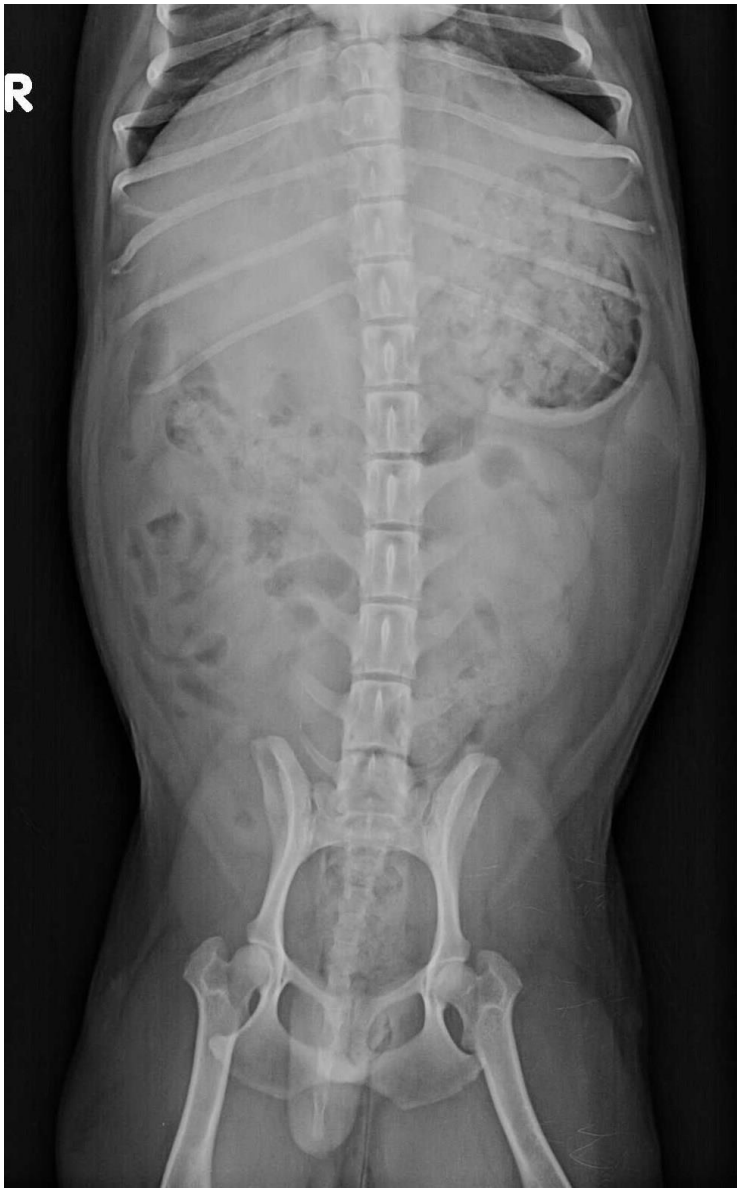
Refinement of the master problem list after the radiographs included diffuse to focal hepatomegaly, loss of radiographic detail in the right cranial abdomen, and an interstitial pattern in the lungs. Differential diagnoses for diffuse hepatomegaly include inflammatory disease or neoplasia, hepatic venous congestion, fat infiltration, cholestasis, cirrhosis,

Figure 1



Right lateral radiographic view of the abdomen on Day 1.

Figure 2



Ventrodorsal radiographic view of the abdomen on Day 1.

amyloidosis, and hepatic storage diseases.[54] Possible causes of the loss of radiographic detail in the right cranial abdomen may include focal hepatomegaly such as localized neoplasia, regenerative nodules or cirrhosis, hepatic abscess or cyst, gallbladder disease, or pancreatitis.[54,106] Possible causes of a diffuse interstitial pattern include an artifact, such as underexposure or end expiratory exposure, or pneumonitis secondary to viral, parasitic, metabolic (uremia or septicemia), inhalant or toxic insults.[105]

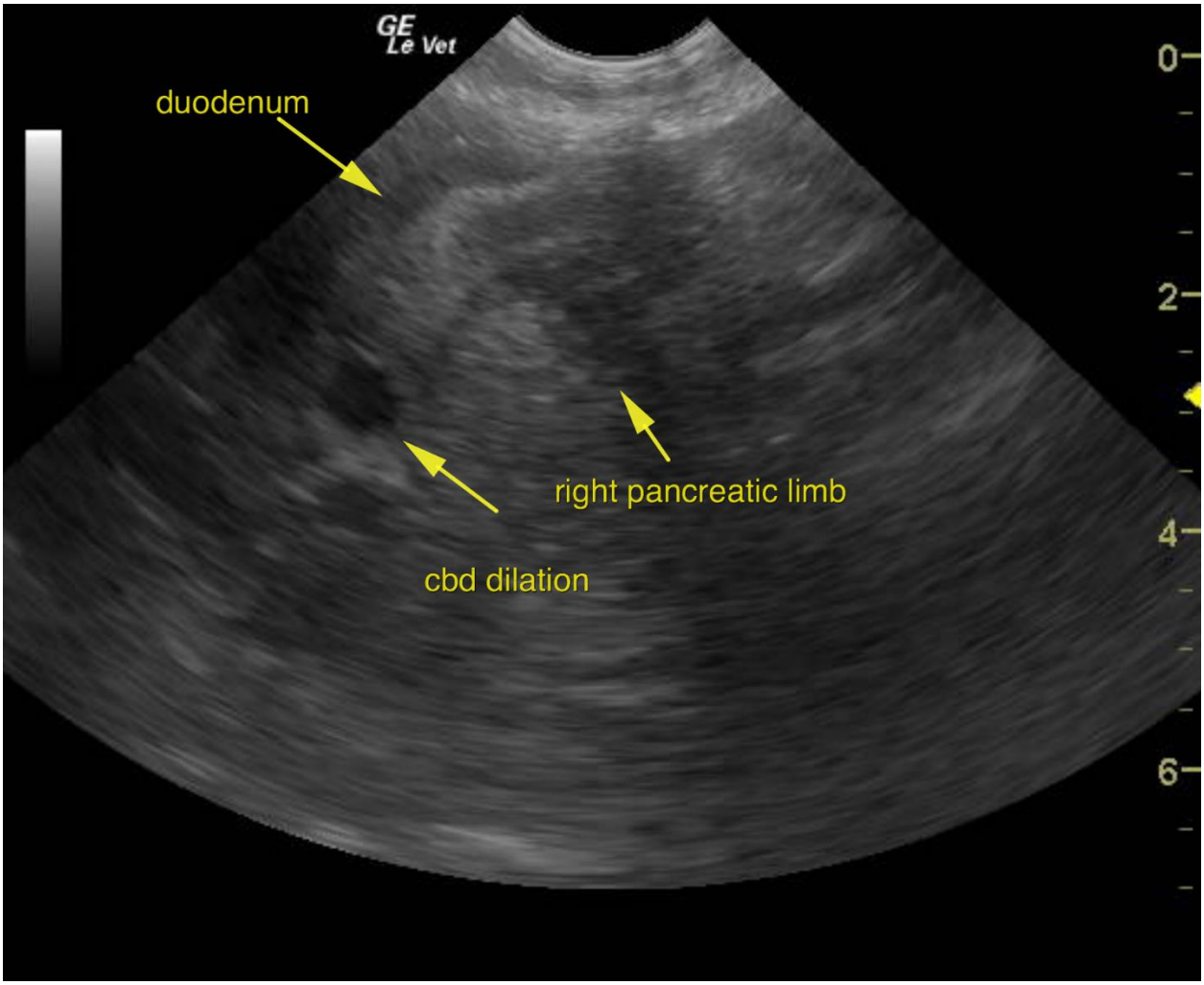
After the initial diagnostic tests were completed, a 20-gauge over the needle catheter[m] was placed in the right cephalic vein. A balanced electrolyte solution 5 ml/kg/hr IV was administered. Once fluid therapy was initiated, a small amount of the bland diet was offered and slowly eaten by the dog. A sample of blood was collected for possible additional diagnostic testing, such as a leptospirosis PCR, toxicology panel, pathology review of the complete blood count by a board-certified veterinary pathologist or a Coombs' test. Patient handling protocols, including avoiding urine, gowning and wearing gloves when handling the dog, washing of hands after handling the dog, and consistent walking outside in a restricted area, were instituted.[39] A combination of ampicillin[n] 20 mg/kg (100 mg) IV q6h, metronidazole[o] 10 mg/kg (50 mg) IV q12h, and enrofloxacin[p] 10 mg/kg (50 mg) IV q24h was administered. The enrofloxacin was diluted with an isotonic crystalloid fluid at ten times the amount of enrofloxacin and administered over 20 minutes via a syringe pump[q]. Based on the previous pain score, hydromorphone[r] 0.1 mg/kg (0.5 mg) IV q4h was administered. Maropitant 1 mg/kg (5 mg) IV q 24h and famotidine 1 mg/kg (5 mg) q24h were also given.

On the second day of hospitalization, the dog was quiet, mildly lethargic, and responsive. The

dog would sit or lie down without whining or vocalization during observation and would stand with a normal posture when approached. On physical examination, her vital signs remained stable with a rectal temperature of 38.6°C (37.5-39.2°C)**,** pulse of 140 beats per minute (110-120 beats per minute), and respiration rate of 36 breaths per minute (15-30 breaths per minute)**.**[90] Her mucous membranes were moist with a capillary refill time of less than two seconds and her skin turgor was normal. The degree of icterus was more prominent in her mucous membranes, scleras, inner pinnae, and ventral abdomen. Her weight remained unchanged at 5.09 kg. No vomiting or diarrhea had occurred in 24 hours. Abdominal palpation continued to show discomfort or pain in the cranial abdomen with a continued pain score of four out of ten despite pain medication.[40] The hydromorphone dose and frequency were increased to 0.2 mg/kg (1 mg) IV q3h due to continued abdominal discomfort. Intravenous fluids, antibiotics, and antiemetics were continued as described.

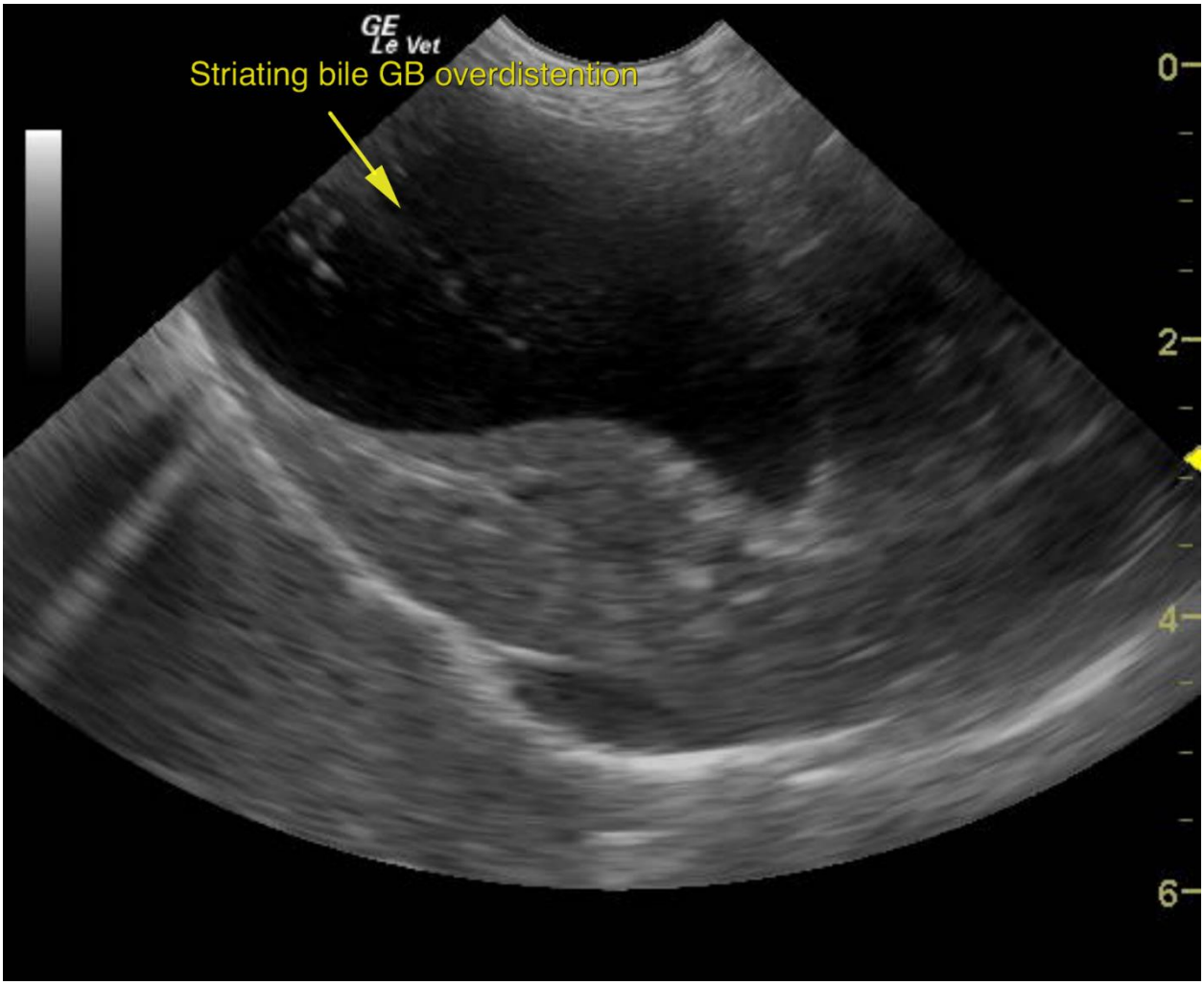
An abdominal ultrasound[s] (Figures 3, 4, 5, and 6) was performed in dorsal recumbency. The ultrasound revealed an enlarged liver with normal hepatic serosal margins and increased parenchyma echogenicity when compared to the spleen.[58] The gallbladder was distended in size with moderate intraluminal debris. The walls of the gallbladder were not thickened or echogenic in appearance. A mildly organized, striated pattern of gallbladder debris was present in the lumen. The cystic duct exiting the gallbladder was dilated. The common bile duct was dilated to 0.5 cm in width and mildly tortuous in appearance. Mild echogenic debris, which moved back and forth with breathing, was noted in the common bile duct lumen along with bile. No free fluid around the gallbladder and no calculi or masses were noted in or adjacent to the common bile duct. When applying pressure to the abdomen in the area of the

Figure 3



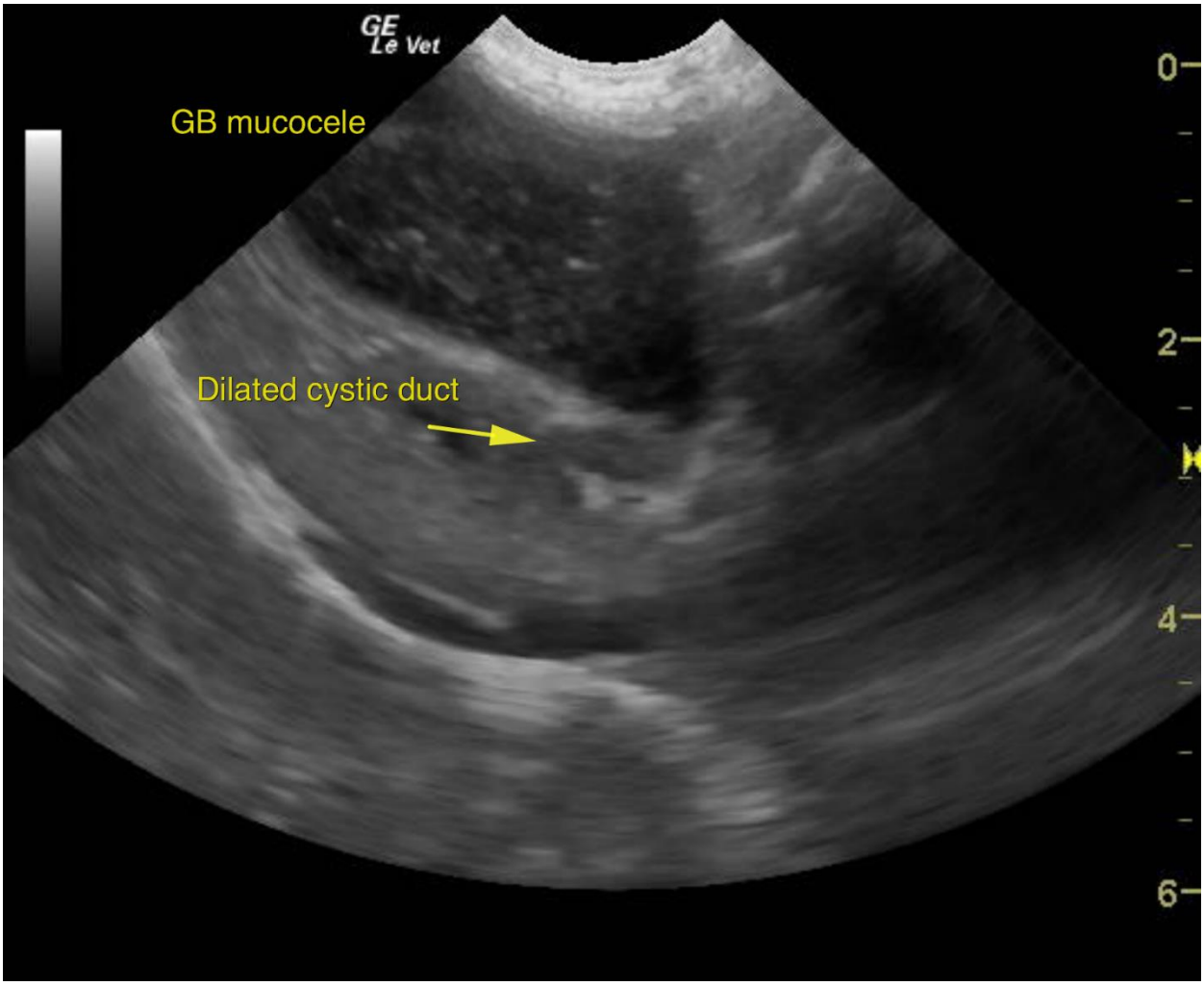
Retrocostal view in right cranial abdomen showing focal hypoechoic pancreas adjacent to descending duodenum and dilated common bile duct at level of duodenal papilla on Day 2.

Figure 4



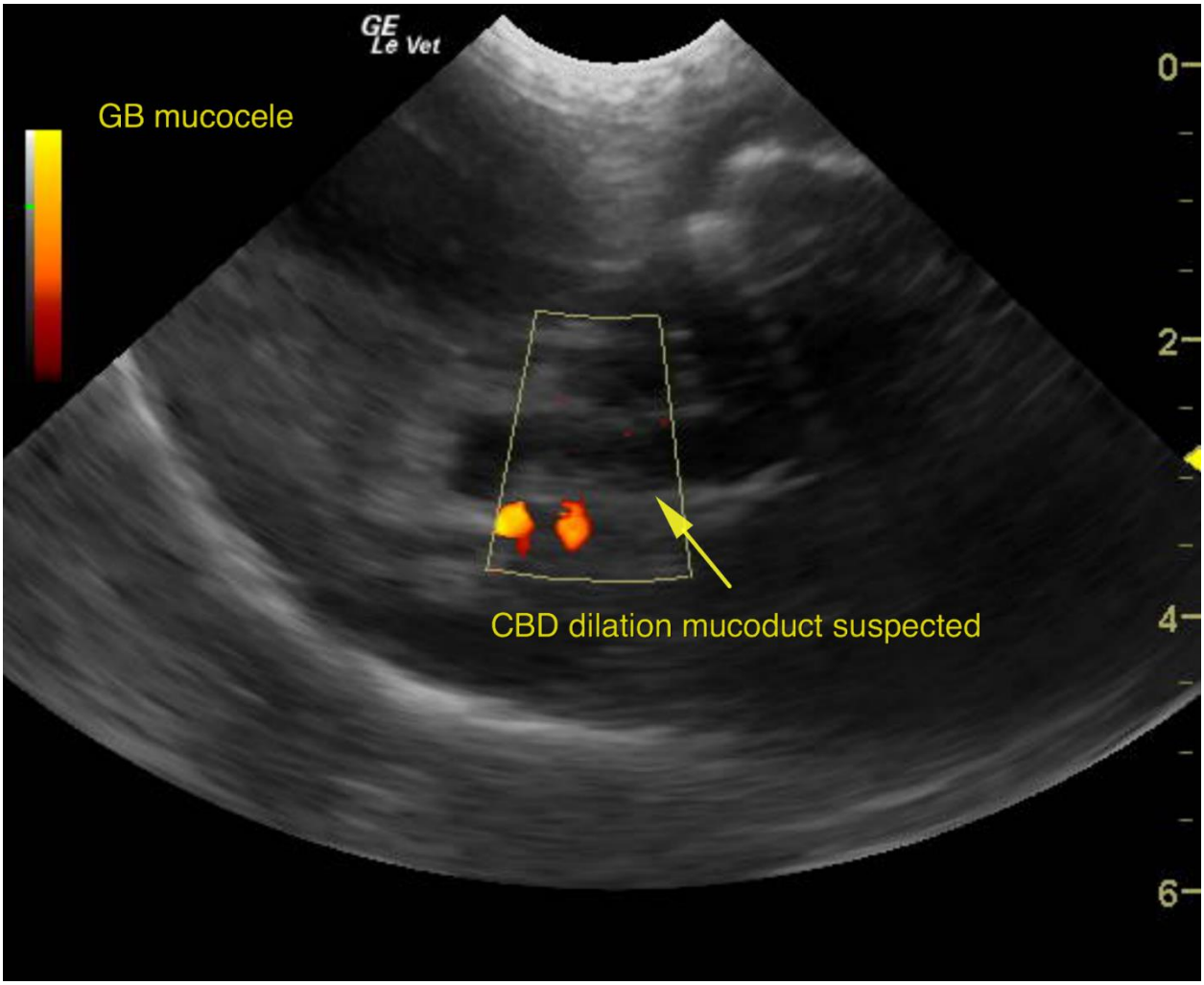
Retrocostal view of distended gallbladder and a non-dependent, striated pattern of intraluminal debris on Day 2.

Figure 5



Retrocostal view of distended gallbladder, non-dependent intraluminal debris, and a dilated cystic duct on Day 2.

Figure 6



Retrocostal view of the dilated common bile duct with Power Doppler showing lack of blood flow within the common bile duct on Day 2.

gallbladder, the dog exhibited signs of pain including movement and increased respiration rate. The discomfort noted when applying pressure to the area of the gallbladder was consistent with a positive sonographic Murphy sign. The volume of the gallbladder was greater than 1 ml/kg of body weight.[5] The appearance of the intraluminal debris was evaluated from a standing position and did not change in appearance.

A small area of hypoechoic parenchyma surrounded by mild hyperechoic omentum was noted in the right pancreatic limb adjacent to the descending duodenum and distal to the common bile duct and duodenal papilla.[59] A sonographic Murphy sign was not noted when pressure was applied to the pancreas. The left and right adrenal glands were normal in size and shape, measuring 1.7 cm length x 0.6 cm width and 1.8 cm length x 0.6 cm width, respectively. The urinary bladder was mild to moderately full with anechoic urine. The walls of the urinary bladder were intact and normal in appearance. The bilateral kidneys were subjectively normal in size and margination with normal echotexture and architectural distinction. The spleen was unremarkable in structure and contour. Intact wall layering and a normal wall thickness were noted in the stomach and small intestine. The lumen of the stomach and small intestine was empty and free of static fluid. The caudal vena cava, aorta and portal vein appeared to be subjectively normal in size with an approximate 1:1:1 ratio.[107] Normal laminar flow was present in the three vessels on color flow Doppler with no evidence of thrombosis. No evidence of peritoneal effusion was found.

The ultrasonographic diagnoses, and refined problem list, included probable focal pancreatitis in the right pancreatic limb, a distended gallbladder with an immature mucocele, a distended to

tortuous common bile duct and mucoduct (mucus or debris present in common bile duct), and common bile duct obstruction.[59,60] A minor potential for other pancreatic disease, such as neoplasia, was considered.[59,60] An obvious cause of common bile duct obstruction was not evident.

An exploratory laparotomy with a possible cholecystectomy and liver biopsy was elected. A biopsy of the pancreas might also be considered if it was grossly abnormal. In preparation for surgery, additional hydromorphone 0.1 mg/kg (0.5 mg) IV was administered for sedation. Once sedated, propofol[t] 4 mg/kg (20 mg) IV was administered slowly for anesthetic induction. A #5.0 endotracheal tube was placed within the trachea and secured using gauze wrap. The endotracheal cuff was inflated to adequate pressure and checked for air leakage. Anesthesia was maintained using a rebreather system with a one liter per minute flow rate of two percent isoflurane[u] and oxygen[v]. Anesthetic monitoring was done with a multi-parameter vital sign monitor[w] which constantly measured heart rate, respiration rate, peripheral capillary oxygen saturation (SPO2), and a three-lead electrocardiogram (ECG) with continuous lead II monitoring. Blood pressure and body temperature were measured every two minutes. Under anesthesia, IV fluid rate was maintained at 5 ml/kg/hr while the vital signs were monitored.[87] Cefazolin[x] 20 mg/kg (112 mg) IV was administered at induction as additional antibiotic therapy. After shaving the ventral abdomen, the dog was placed in dorsal recumbency on the surgical table. The ventral abdomen was prepared by three scrubs each of povidone-iodine[y] followed by alcohol[z].

During surgery, a ventral midline incision was made through the linea alba. The liver was

subjectively enlarged but the liver parenchyma appeared grossly normal. The gallbladder was located which was distended in size and moderately firm on palpation. The integrity of the gallbladder wall appeared to be intact with no evidence of surrounding tissue reaction, adhesions, or bile leakage. The common bile duct was located and was mildly distended in appearance. The gallbladder was separated from the visceral peritoneum and liver using careful dissection with Metzenbaum scissors[aa] and digital manipulation. Once free from the surrounding tissue, gentle manual pressure was applied to the common bile duct while monitoring it for increases in size. Bile was easily expressed from the common bile duct toward the duodenum with no changes in its diameter, indicating the common bile duct was patent. Next, the cystic duct and artery were located adjacent to each other. Two Kelly hemostat forceps[bb] were placed in proximal and distal orientation along both structures. Two ligatures using 2-0 polydioxanone[cc] suture were placed proximal to the hemostats to ligate the cystic duct and artery, and the gallbladder was removed by excising between the two hemostat forceps. After removing the remaining hemostat forcep, no bleeding or leakage from the ligated cystic duct and artery was noted prior to placement back into the abdominal cavity.

Following removal of the gallbladder, a wedge biopsy of the right middle liver lobe was obtained. Two Kelly hemostat forceps were placed on the margin of the right middle liver lobe to make a V shaped piece of liver tissue approx. 1 cm x 1 cm in size. The hemostat forceps were left in place for several minutes and the wedge shaped piece of liver tissue was removed. The hemostat forceps were again left in place for several minutes before removing them from the liver. Mild oozing from the area of biopsy was noted once the hemostat forceps were removed, so guillotine sutures using 2-0 polydioxanone were placed around the margin of the biopsy for

further hemostasis. After placement of the sutures, no further bleeding or oozing was noted. The ligated cystic duct and artery as well as common bile duct were examined again with no signs of bleeding or leakage. The remainder of the abdomen was examined with no structural abnormalities found.

Closure of the abdominal incision occurred in three layers. The linea alba was closed using 2-0 polydioxanone in a simple continuous pattern. The subcutaneous tissue was closed using 2-0 polydioxanone in a simple continuous pattern. The skin was closed using 3-0 polydioxanone[dd] in an intradermal pattern. The surgery took approximately one and one quarter hours. Vital signs remained stable during surgery and in the immediate postoperative period (Table 8). Vital signs were continuously monitored after surgery until the body temperature was normal.

The postoperative fluid rate was decreased to 3 ml/kg/hr, a rate consistent with the daily fluid requirement of the dog.[86] The endotracheal tube was removed once the dog began swallowing. Hydromorphone 0.2 mg/kg (1 mg) IV q3h was continued for pain control. Following a second dose two hours following the initial injection, cefazolin 20 mg/kg (112 mg) IV q8h was continued for the remainder of hospitalization. Ampicillin was discontinued but enrofloxacin and metronidazole were continued as previously described. A small amount of bland diet was offered later in the day and was eaten. Overnight hospitalization for continued monitoring, treatment and supportive care was elected.

On the third day of hospitalization, the dog was bright, alert and responsive. Vital signs remained normal with a rectal temperature of 38.6^{o}C (37.5–39.2^{o}C), a heart rate of 100 beats per minute (110-120 beats per minute), a respiration rate of 36 breaths per minute (15-30

Table 8: Intraoperative and postoperative vital signs, Day 2 (**Bold** indicates outside of reference range)

Vital sign	Intraoperative Time (minute)						Postoperative time (minute)					Normal[90,97]
	0	15	30	45	60	75	0	15	30	45	60	
Temp (°C)	38.6	37.8	**37.1**	**36.7**	**36.4**	**36.3**	**36.3**	**36.7**	**37.2**	37.5	38.0	37.5–39.2
Heart rate	**100**	112	110	110	**105**	**100**	**100**	120	**124**	120	**126**	110-120
Resp rate	20	**10**	**10**	**12**	**10**	**12**	**10**	24	20	22	24	15-30
MM	pink	pink	pink	pink	pink	pink	pink	pink	pink	pink	pink	pink
CRT(sec)	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
SpO_2(%)	98	98	98	98	98	99	96	**97**	**97**	**97**	**97**	98-100
MAP(mmHg)	102	95	100	105	102	100	100	110	112	115	118	85-120

breaths per minute).[90] Her capillary refill time was normal at less than two seconds. Normal blood pressure was noted as the MAP was 118 mmHg (85-120 mmHg).[97] The ventral midline incision was mildly inflamed with no discharge. When the abdomen was palpated, focal mild discomfort was present around the incision but generalized pain in the cranial abdomen was not perceived. A pain score of two out of ten was assigned to the dog. A small amount of bland diet was offered and eaten with a good appetite. No vomiting was noted after eating. Since the dog's appetite was normal, IV medications were discontinued. Oral antibiotics were prescribed: cefpodoxime[ee] 5 mg/kg (25 mg) PO q24h for 28 days, metronidazole[ff] 10 mg/kg (50 mg) PO q12h for 28 days, and enrofloxacin[gg] 6.8 mg/kg (34 mg/kg) PO q24h for 28 days. Meloxicam[hh] 0.2 mg/kg (1 mg) PO q24h for 7 days and tramadol[ii] 5 mg/kg (25 mg) PO q8h for 7 days were prescribed and administered as oral anti-inflammatory and pain medications, respectively. Omeprazole[jj] 1 mg/kg (5 mg) PO q24h was prescribed as an antacid. SAMe[kk] 20 mg/kg (100 mg) PO q 24h for 30 days was also prescribed.

The dog was discharged at the end of the third day of hospitalization with the prescribed medications and the bland diet. A follow-up physical examination was scheduled for one month after surgery to recheck the bloodwork but the owner was advised to have the pet rechecked sooner if abnormal clinical signs, such as vomiting, diarrhea, lethargy, or continued icterus, were noted. Follow-up phone conversations with the owner on first and fourth day after discharge indicated that the dog was recovering normally at home. The owner stated that the dog was mildly restless during the first day after discharge but was acting normally on the second day. The owner reported that the dog's appetite had been normal with no vomiting and normal stool. Normal water intake and urination were also reported. Medications were being

given as directed.

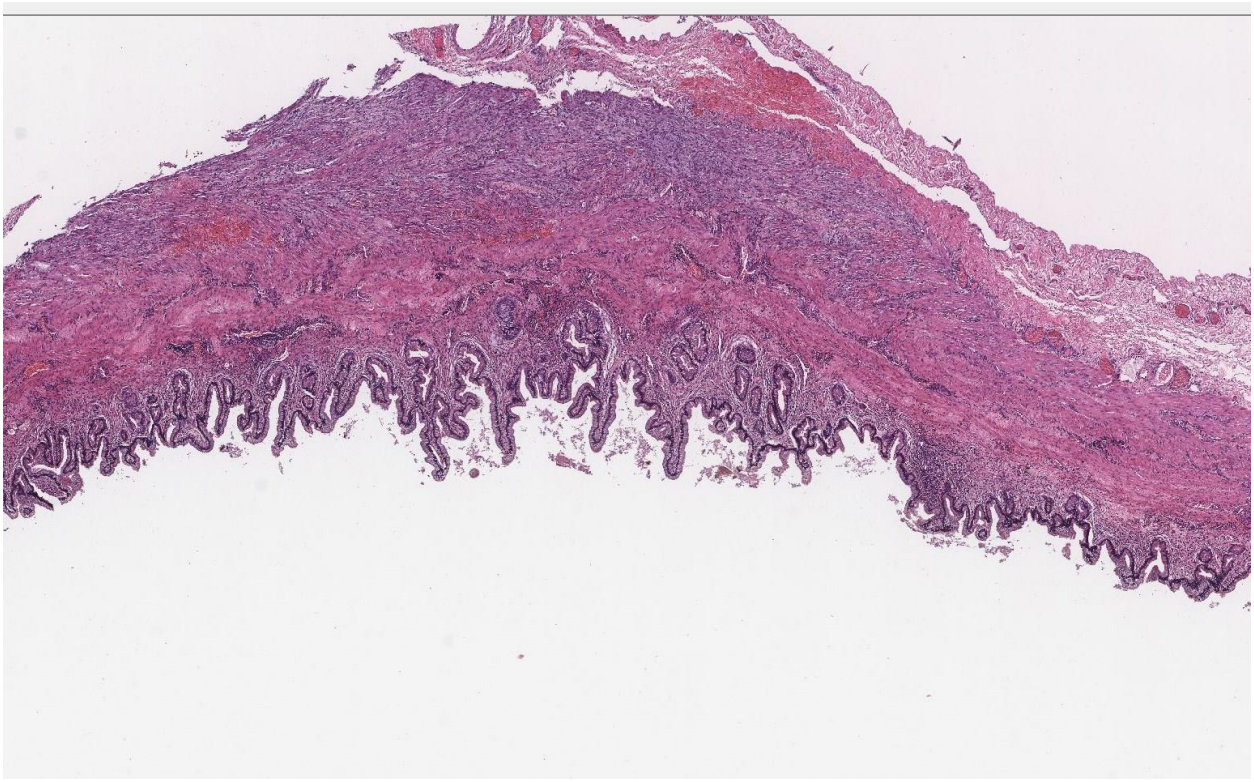
The gallbladder and the liver biopsy were submitted to an outside laboratory for histopathology. The histopathologic diagnosis confirmed the presence of a gallbladder mucocele with secondary changes in the liver that were attributed to chronic bile stasis or extrahepatic biliary obstruction (Figure 7). The described changes in the pathology report could be seen in the histopathology micrographs (Figures 8, 9 and 10). Copper staining of the liver tissue did demonstrate moderate amounts of centrolobular copper accumulation in zones 2 and 3. The primary causes of copper accumulation within liver tissue, which is often seen in areas of vacuolar degeneration, may include cholestasis and breed-associated hereditary copper toxicosis.[37]

The dog presented 30 days after surgery for follow-up bloodwork and physical examination. The owner reported that the dog was acting normally with good appetite, was drinking normal amounts of water with no increase in water intake or urination, and was having normal bowel movements. On physical examination, the dog was bright, alert, and responsive. Her vital signs were normal with a rectal temperature of 38.7^{o}C (37.5^{o}C-39.2^{o}C)**,** pulse of 136 beats per minute (110-120 beats per minute), and respiration rate of 40 breaths per minute (15-30 breaths per minute)**.**[90] Her mucous membranes were moist with a capillary refill time of less than two seconds. No signs of icterus were noted in the mucous membranes, scleras, inner pinnae or ventral abdomen. The ventral midline incision had healed normally with no signs of inflammation, infection or suture reaction. Abdominal palpation was soft with no palpable abnormalities or discomfort. Her body weight was 5.3 kg, which was slightly heavier than her

Figure 7

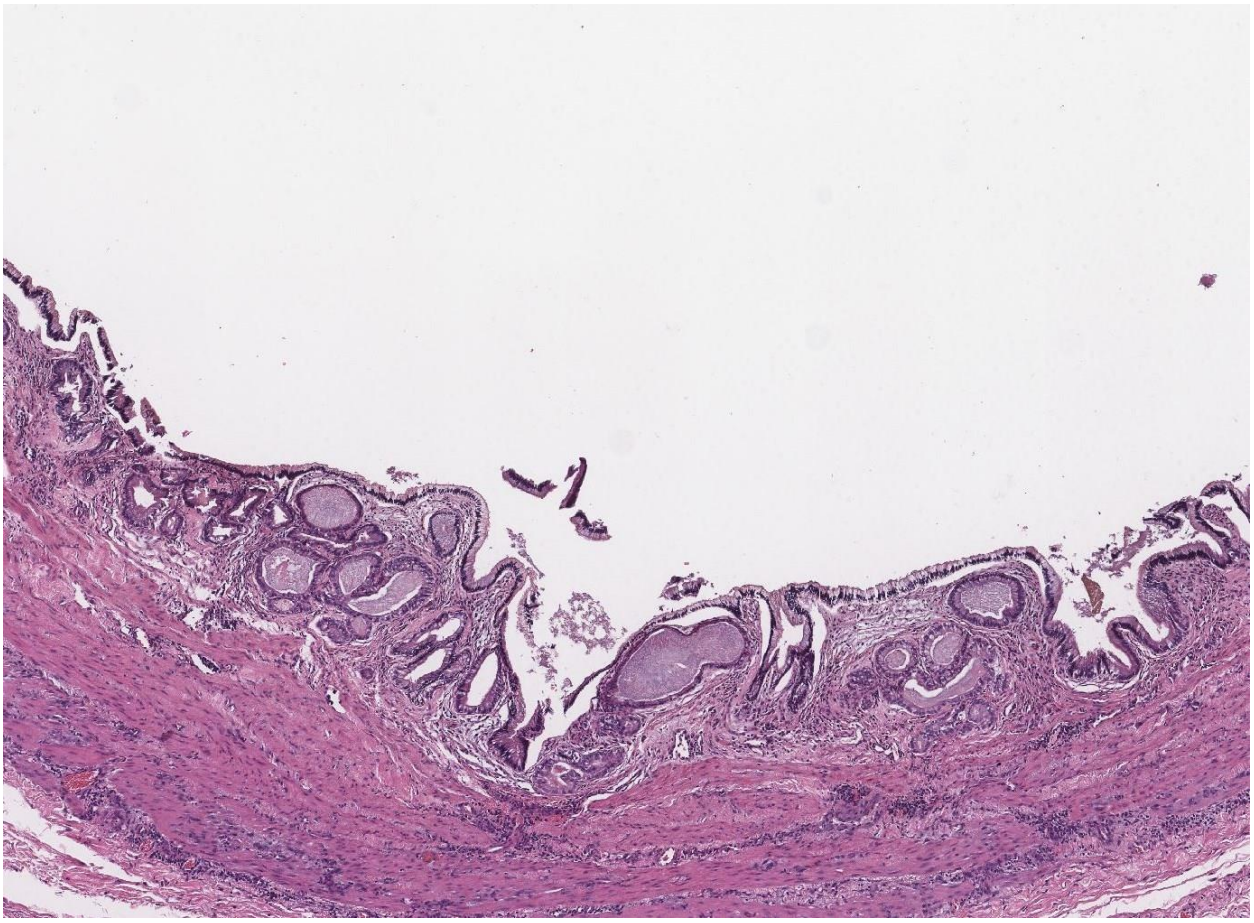
Histopathology: Gallbladder and Liver
Description:
Within the gallbladder, the epithelium was diffusely hyperplastic with multifocal, narrow, short, interconnecting villous projections, consistent with a reactive increase in epithelial cell numbers and thickening of the gallbladder wall. Moderate numbers of perivascular lymphocytes and plasma cells were noted within the submucosa, indicative of an inflammatory process. Large numbers of small blood vessels and fibroblasts infiltrated and replaced the gallbladder wall, indicative of replacement of the gallbladder wall with connective tissue or gallbladder wall repair. Within the liver, the portal triads were often in close apposition to the central veins, consistent with lobular atrophy or collapse which is often seen with hepatic tissue damage. The portal regions were expanded by mild amounts of fibrosis, consistent with tissue injury and a reparative or reactive process, and increased numbers of bile ducts, a reactionary process generally seen in cholestatic disease. Hepatocytes were also mildly distended by clear distinct to lacy granular cytoplasmic granules which contained glycogen and lipid, consistent with vacuolar degeneration, and moderate amounts of centrolobular pigment, indicative of retained bile within the hepatocytes. Zone 2 to 3 hepatocytes contain moderate amounts of centrolobular pigment. Copper staining did demonstrate moderate amounts of centrolobular copper within the liver and this pigment tends to be present in areas of vacuolar degeneration.
Microscopic findings:
1. Moderate diffuse chronic villous hyperplasia (mucocele) 2. Moderate multifocal chronic lymphoplasmacytic cholecystitis 3. Moderate multifocal lobular collapse , biliary hyperplasia and centrolobular hepatocellular pigment

Figure 8



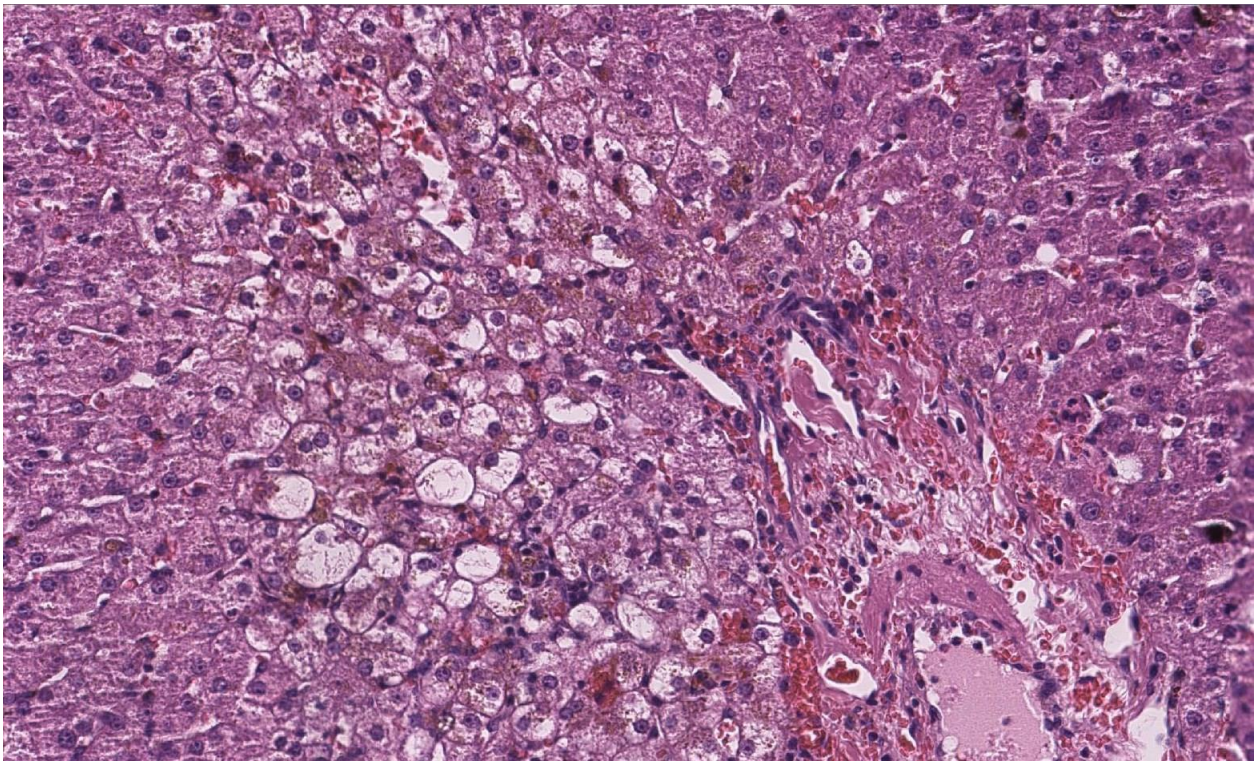
Histopathology micrograph of the thickened gallbladder wall with villous hypertrophy.

Figure 9



Histopathology micrograph of the gallbladder wall with villous hypertrophy. The cystic structures within the epithelium contain thickened bile.

Figure 10



High magnification micrograph of the liver showing vacuolar hepatopathy.

initial body weight, and her BCS was 5/9.[91]

A complete blood count, chemistry panel, and urinalysis were submitted to an outside laboratory[ll] for evaluation (Tables 9, 10, and 11). The complete blood count and urinalysis were unremarkable with resolution of the monocytosis and bilirubinuria. The chemistry panel revealed that the hyperbilirubinemia and elevations of ALT, AST, and GGT had resolved. ALKP levels had decreased from the initial chemistry panel but remained elevated. Hypertriglyceridemia and hypercholesterolemia were also present.

Potential causes of the continued ALKP elevation included continued intrahepatic cholestasis, resolving or continued vacuolar hepatopathy, reactive hepatopathy secondary to the hyperlipidemia or an endocrinopathy.[43] Differential diagnoses for the hypertriglyceridemia and hypercholesterolemia include hepatic insufficiency, continued pancreatitis or intrahepatic cholestasis, idiopathic or breed-associated elevation, or an endocrinopathy, specifically hyperadrenocorticism.[104] A post prandial sample resulting in elevated triglyceride levels was considered less likely as the owner had not fed the dog prior to presentation. Antibiotics were discontinued due to the resolution of the elevated ALT, AST and TBIL**.** SAMe was continued as previously described. Ursodeoxycholic acid[mm] 5 mg/kg (25 mg) PO q12h was added to the treatment protocol. The owner was advised of the severe elevations in the serum triglycerides and to monitor for clinical signs associated with the hypertriglyceridemia. The diet was changed from a bland diet to a low fat diet[nn] which contained 19 g/Mcal of fat.[24] A recheck of liver values and serum lipid levels after a confirmed 12-hour fast was recommended in 60 days. The owner was advised to have the pet examined sooner if any abnormalities were noted.

Table 9: Complete Blood Count, Day 30 (**Bold** indicates outside reference range)

CBC	Result	Reference Range	Units
Hematocrit	49.4	36-60	%
Hemoglobin	17.3	12.1-20.3	g/dL
RBC	7.47	4.8-9.3	10^6/uL
MCHC	35.1	30-38	g/dL
MCV	66.2	58-79	fL
MCH	23.2	19-28	pg
WBC	12.1	4.0-15.5	10^3/uL
Neut	70	60-77	%
Lymphs	19	12-30	%
Monos	6	3-10	%
Eos	5	2-10	%
Baso	0	0-1	%
Neut Bands	0	0-3	%
Abs Neut	8470	2060-10600	/uL
Abs Lymphs	2057	690-4500	/uL
Abs Monos	726	0-840	/uL
Abs Eos	605	0-1200	/uL
Abs Neut Bands	0	0-300	/uL
Abs Baso	0	0-150	/uL
Platelets	323	170-400	10^3/uL
Platelet (est)	Adequate		
Blood parasites	None seen		

Table 10: Chemistry Panel, Day 30 (**Bold** indicates outside reference range)

Chemistry Profile	Result	Reference Range	Units
Albumin	3.8	2.7-4.4	g/dL
ALKP	**520**	**5-131**	**U/L**
ALT	111	12-118	U/L
AST	30	15-66	U/L
AMYL	826	290-1125	U/L
BUN	13	6-31	mg/dL
Ca	10.6	8.9-11.4	mg/dL
Chloride	115	102-120	mEq/L
CHOL	**390**	**92-324**	**mg/dL**
CK	130	59-895	U/L
CREA	0.8	0.5-1.6	mg/dL
GGT	5	1-12	U/L
Glucose	89	70-138	mg/dL
Mg	2.1	1.5-2.5	mEq/dL
PHOS	4.1	2.5-6.0	mg/dL
Potassium	3.8	3.6-5.5	mEq/dL
Sodium	142	139-154	mEq/dL
TBIL	0.2	0.1-0.3	mg/dL
Total Protein	6.9	5.0-7.4	g/dL
TRIG	**1024**	**29-291**	**mg/dL**
Globulin	3.1	1.6-3.6	g/dL
Ca (Corr)	10.5		
A/G ratio	1.0	0.8-2.0	Ratio
B/C ratio	16	4-27	Ratio
Na/K ratio	37		Ratio
Precision PSL	110	24-140	

Table 11: Urinalysis, Day 30 (**Bold** indicates outside of reference range)

Urinalysis	Result	Reference Range
Color	Yellow	
Clarity	Clear	
Specific Gravity	1.035	1.015-1.050
Glucose	Negative	Negative
Bilirubin	Negative	Negative
Ketones	Negative	Negative
Blood	Negative	Negative
pH	7.5	6.0-7.5
Protein	Trace	Negative
WBC	0-2	0-3
RBC	0-2	0-3
Bacteria	None seen	None
Epi Cell	None seen	0-3
Mucus	Negative	Negative
Casts	None seen	None
Crystals	None seen	None
Urobilinogen	Negative	Negative

Follow-up bloodwork was performed on day 90 following surgery. No abnormalities were reported by the owner at home. The owner had been compliant with feeding the low fat diet exclusively and had withheld food from the dog for 12 hours. On physical examination, the dog was bright, alert and responsive. Her vital signs were normal with a rectal temperature of 38.4°C (37.5°C-39.2°C), pulse of 120 beats per minute (110-120 beats per minute), and respiration rate of 32 breaths per minute (15-30 breaths per minute).[90] Her mucous membranes were moist with a capillary refill time of less than two seconds. Abdominal palpation was unremarkable with no palpable abnormalities or evidence of discomfort. No signs of icterus were noted. Her body weight remained unchanged at 5.3 kg and her BCS was 5/9.[91] A complete blood count, chemistry panel, urinalysis and thyroxine level were submitted to an outside laboratory for evaluation (Tables 12, 13, and 14).

The complete blood count, chemistry panel, and urinalysis from day 90 after the cholecystectomy revealed a continued but improved elevation of ALKP and increased serum triglyceride levels from the previous chemistry panel. Serum cholesterol levels were also mildly elevated. The other liver enzymes were within normal limits with normal serum bilirubin levels. Mild bilirubinuria was present on the urinalysis while the urine specific gravity was greater than 1.020.

Differential diagnoses for the persistent ALKP elevation continued to include hepatic cholestasis, resolving or continued vacuolar hepatopathy, reactive hepatopathy secondary to the hyperlipidemia, pancreatitis and, less likely, PDH.[43] Possible causes of the persistent hypertriglyceridemia and mild hypercholesterolemia continued to include hepatic insufficiency, unresolved mild pancreatitis, cholestasis, idiopathic or breed-associated elevation, and, less

Table 12: Complete Blood Count, Day 90 (**Bold** indicates outside of reference range)

CBC	Result	Reference Range	Units
Hematocrit	55	36-60	%
Hemoglobin	19.7	12.1-20.3	g/dL
RBC	8.1	4.8-9.3	10^6/uL
MCHC	35.8	30-38	g/dL
MCV	68	58-79	fL
MCH	24.3	19-28	pg
WBC	10.9	4.0-15.5	10^3/uL
Neut	73	60-77	%
Lymphs	18	12-30	%
Monos	7	3-10	%
Eos	2	2-10	%
Baso	0	0-1	%
Neut Bands	0	0-3	%
Abs Neut	7957	2060-10600	/uL
Abs Lymphs	1962	690-4500	/uL
Abs Monos	763	0-840	/uL
Abs Eos	218	0-1200	/uL
Abs Neut Bands	0	0-300	/uL
Abs Baso	0	0-150	/uL
Platelets	399	170-400	10^3/uL
Platelet (est)	Increased		
Blood parasites	None seen		

Table 13: Chemistry Panel, Day 90 (**Bold** indicates outside of reference range)

Chemistry Profile	Result	Reference Range	Units
Albumin	4.4	2.7-4.4	g/dL
ALKP	**441**	5-131	U/L
ALT	110	12-118	U/L
AST	62	15-66	U/L
AMYL	530	290-1125	U/L
BUN	16	6-31	mg/dL
Ca	10.9	8.9-11.4	mg/dL
Chloride	102	102-120	mEq/L
CHOL	**359**	92-324	mg/dL
CK	530	59-895	U/L
CREA	0.6	0.5-1.6	mg/dL
GGT	2	1-12	U/L
Glucose	78	70-138	mg/dL
Mg	2.5	1.5-2.5	mEq/dL
PHOS	3.9	2.5-6.0	mg/dL
Potassium	4.7	3.6-5.5	mEq/dL
Sodium	148	139-154	mEq/dL
TBIL	0.3	0.1-0.3	mg/dL
Total Protein	6.9	5.0-7.4	g/dL
TRIG	**2244**	29-291	mg/dL
Globulin	2.5	1.6-3.6	g/dL
Ca (Corr)	10.6		
A/G ratio	1.8	0.8-2.0	Ratio
B/C ratio	27	4-27	Ratio
Na/K ratio	31		Ratio
Precision PSL	87	24-140	
T4	1.2	0.8-3.5	ug/dL

Table 14: Urinalysis, Day 90 (**Bold** indicates outside of reference range)

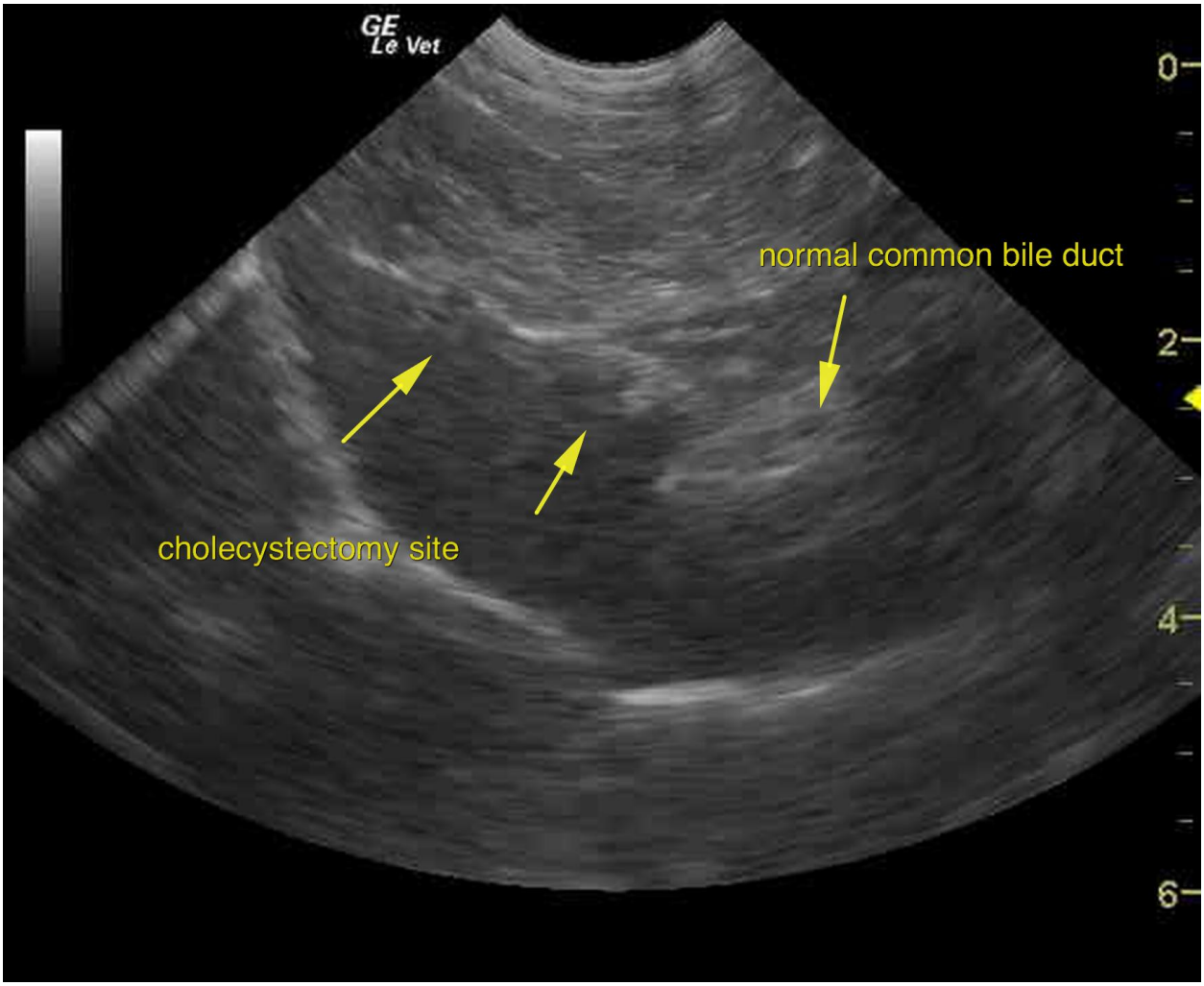
Urinalysis	Result	Reference Range
Color	Yellow	
Clarity	Cloudy	
Specific Gravity	1.028	1.015-1.050
Glucose	Negative	Negative
Bilirubin	**1+**	Negative
Ketones	Negative	Negative
Blood	Negative	Negative
pH	7.0	5.5-7.0
Protein	Trace	Negative
WBC	0	0-3
RBC	0	0-3
Bacteria	None seen	None
Epi Cell	0-1	0-3
Mucus	Negative	Negative
Casts	None seen	None
Crystals	None seen	None
Urobilinogen	Negative	Negative

likely, PDH.[104] The mild bilirubinuria on the urinalysis was not considered to be clinically significant.[9]

A repeat abdominal ultrasound (Figures 11, 12 and 13) was performed. The ultrasound showed an overall increased hepatic echogenicity with a marked increase in echogenicity around the cholecystectomy site. The liver contour was slightly rounded in appearance with mildly increased echogenicity of the portal vasculature, otherwise described as increased portal markings.[58] The common bile duct was normal in appearance. The bilateral adrenal glands were consistent in size and appearance to the initial ultrasound, both measuring 0.6 cm in width. The focal area of pancreatitis appeared to have resolved. The kidneys, spleen, gastrointestinal tract, and urinary bladder were similar in appearance compared to the previous ultrasound. No enlarged abdominal lymph nodes or peritoneal effusion was found.

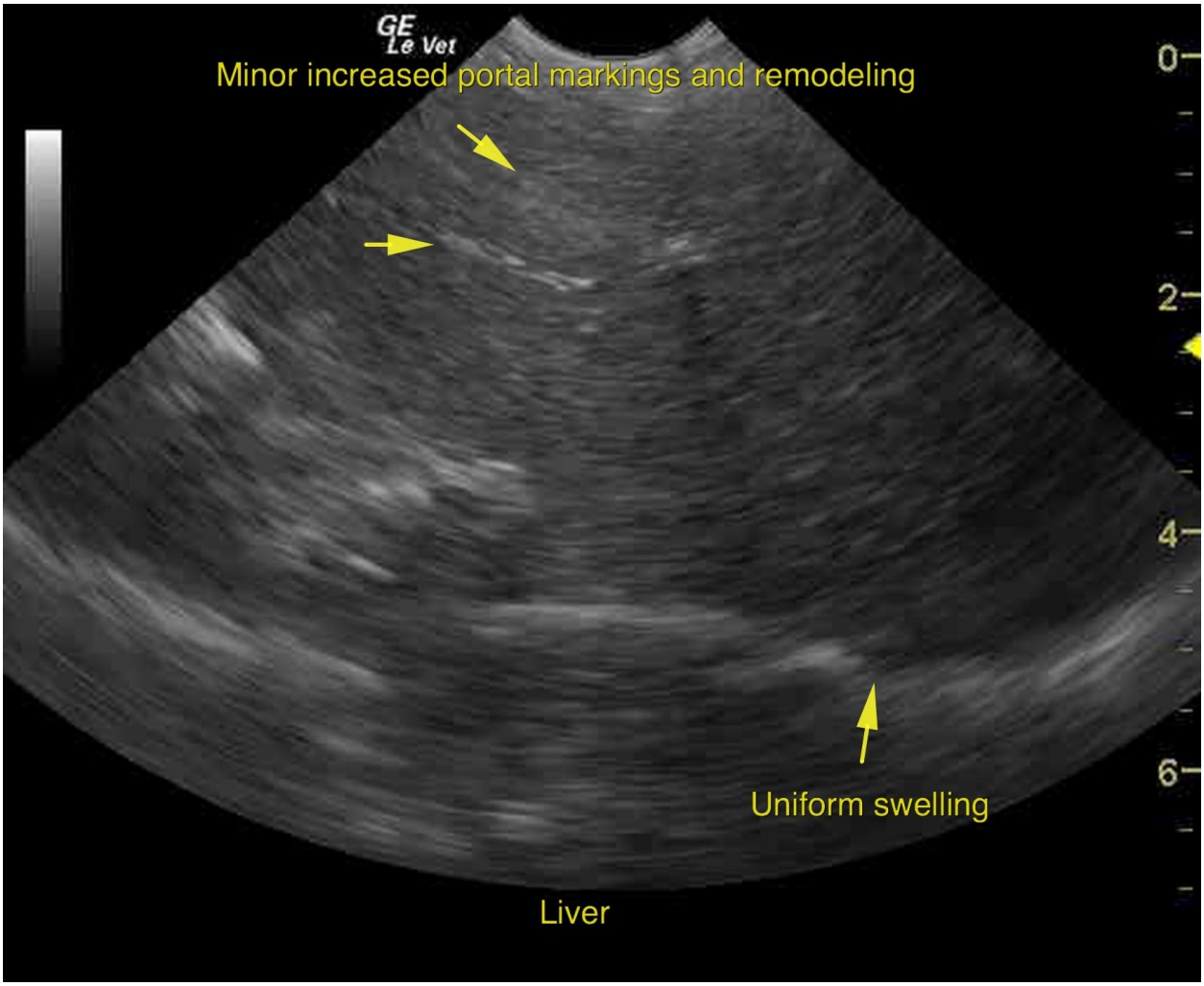
Preprandial and postprandial bile acids (Table 15) and a low dose dexamethasone suppression test (LDDST)(Table 16) were elected to assess liver functionality and determine if pituitary dependent hyperadrenocorticism was the cause of the continued ALKP elevation and hyperlipidemia, respectively. The preprandial and postprandial bile acid levels were within normal limits. The elevated cortisol levels in the initial venous blood sample was most attributable to stress.[108] Adequate suppression of cortisol levels following the administration of dexamethasone was present on the LDDST.[18] SAMe, ursodeoxycholic acid, and the low fat diet were continued as previously described. Supplementation with fish oil containing EPA and DHA and chitosan were also added to the treatment regimen. Consistent monitoring of the triglyceride levels and liver enzymes was highly recommended.

Figure 11



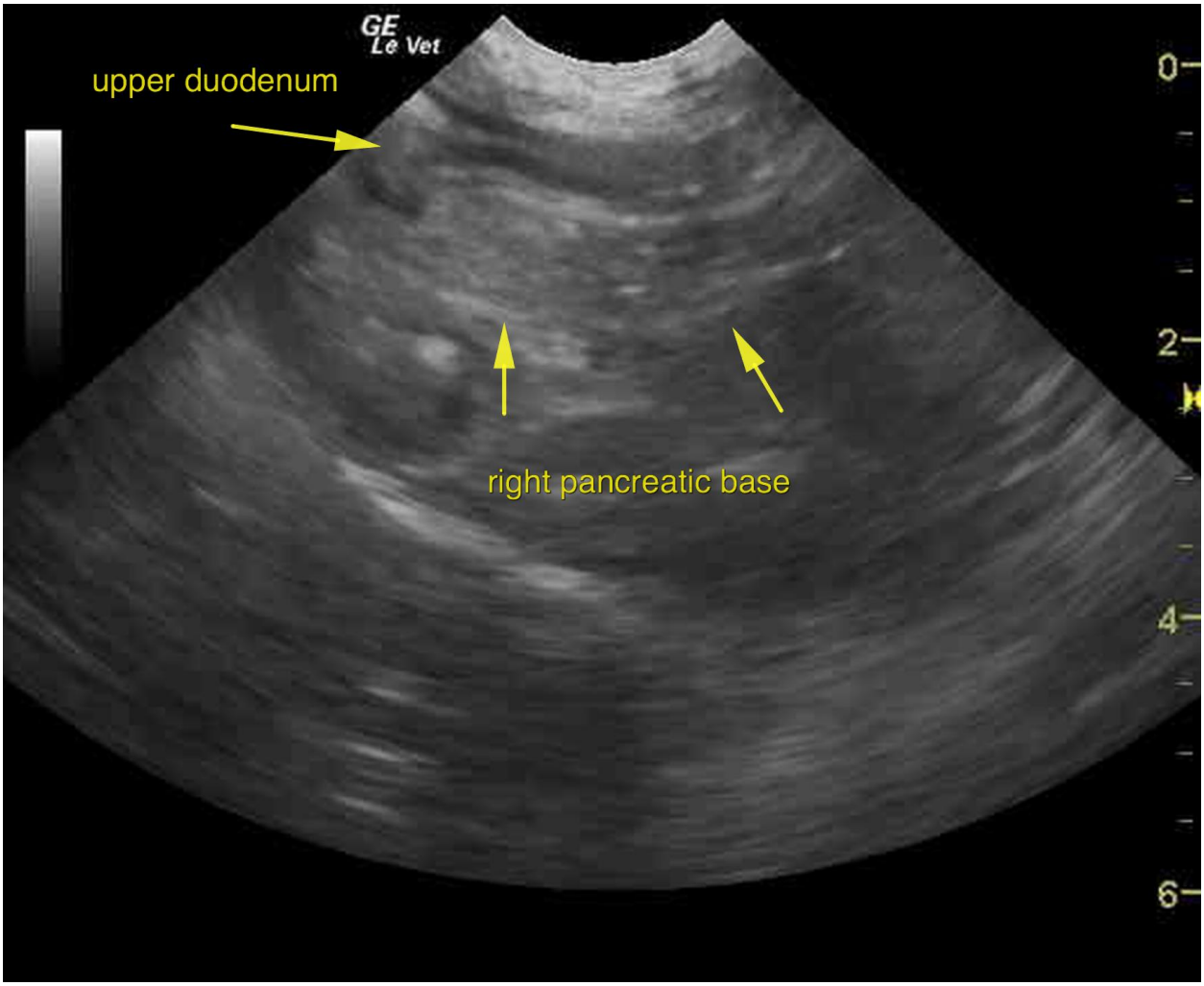
Retrocostal view of the right to mid liver which shows increased echogenicity adjacent to the cholecystectomy site and a normal common bile duct on Day 90.

Figure 12



Sub-xiphoid transverse view of the liver showing mild hepatic enlargement as indicated by slightly rounded serosal margins and minor increased echogenicity of the portal vasculature on Day 90.

Figure 13



Retrocostal view of the right pancreatic limb showing uniform parenchyma on Day 90.

Table 15: Bile acids, Day 90 (**Bold** indicates outside of reference range)

	Result	Reference Range
Pre feeding	5.5	<10 umol/L
2 hour post feeding	18.8	<20 umol/L

Table 16: Low dose dexamethasone suppression test, Day 90 (**Bold** indicates outside of reference range)

Sample	Cortisol level	Reference range
Initial	**12.4**	2-6 ug/dL
4 hour	<1.0	None
8 hour	<1.0	<1.4 ug/dL

Discussion

The presenting abnormalities at the initial examination were non-specific as icterus was not evident. There was no evidence of orolaryngeal, nasal, musculoskeletal disease of the skull, or respiratory disease as potential causes of anorexia on physical examination.[92] Therefore, the cause of the anorexia was most likely originating from the abdominal cavity which corresponded with mild abdominal discomfort. The most probable cause of the dehydration was decreased water intake in combination with natural fluid loss as the owner did not report increased urination or consistent vomiting or diarrhea.[95] Although heart and lung disease could not be ruled out, the mild tachycardia and tachypnea was consistent with mild elevations of the heart and respiratory rate seen with discomfort, pain, or anxiety as there was no detectable murmur, arrhythmia, or abnormal lung sounds on thoracic auscultation.[94] Gastrointestinal inflammation or pancreatitis secondary to dietary indiscretion were reasonable differential diagnoses at the time of the initial presentation.

The SNAP cPL test was elected as a screening test for pancreatitis as the clinical signs were similar to those that may be seen with the disease. The owner was advised of the limitations of a sole SNAP cPL test in the diagnosis of pancreatitis as false positive results and false negative results may occur.[49] When the normal results of the SNAP cPL test were confirmed, pancreatitis was considered to be a less likely cause of the clinical signs but could not be definitively ruled out. The initial conservative treatment for suspected gastroenteritis secondary to dietary indiscretion was logical with limited diagnostics and non-specific clinical signs. The balanced electrolyte solution was administered SQ to provide a large quantity of fluid which would be

gradually absorbed to prevent further fluid loss and possibly alleviate mild dehydration.[87] Famotidine was the only injectable antacid available at presentation and was administered to reduce gastrointestinal acid production.[71] Maropitant was prescribed for treatment of both central and peripheral causes of nausea or vomiting and to address suspected visceral pain.[74,75]

After initial conservative treatment failed to alleviate the underlying medical problem, dehydration, icterus, weight loss and definitive abdominal discomfort were the primary abnormalities noted on physical examination. The suspected cause of the dehydration continued to be decreased water intake in combination with natural fluid loss as the owner did not report increased urination or consistent vomiting or diarrhea.[95] Although the pale pink mucous membranes were not consistent with obvious anemia, hemolysis, hepatobiliary disease and posthepatic obstruction were the primary diseases that were considered as possible causes of the icterus. The weight loss was most attributable to decreased caloric consumption based on the history of decreased appetite, previous and current feedings of well-balanced diets, lack of consistent gastrointestinal signs such as vomiting or diarrhea, and no reported abnormal urination.[92,99]

Visceral pain was considered to be most likely as the pain appeared to be indistinct and difficult to localize with no evidence of trauma, neurologic deficits in the rear limbs, or signs of musculoskeletal disease.[40] The degree of pain was assessed using a numerical rating scale, a semi-objective assessment of pain that comprises multiple categories from which to evaluate the patient.[40] The appearance of the eyes, interactive behaviors, physiologic parameters and reaction to palpation were taken into account when the degree of pain was determined.[40] A subjective pain score of four, or moderate pain, was given to the patient.

The initial complete blood count revealed a low normal hematocrit and a mild monocytosis. Although an anemia was not present, the low normal hematocrit could have suggested active hemolysis that was still in the reference range. A manual PCV was run to correlate with the automated hematocrit, while the manual differential was used to assess for possible blood parasites or abnormal red blood cell morphology for further evaluation of potential hemolysis.

To further investigate whether autoimmune disease was present, a saline agglutination test was also performed as a screening test for the presence of antibodies on the outside of the red blood cells.[32,33] To perform this test, a drop of anticoagulated blood, obtained from the collection tube containing ethylenediaminetetraacetic acid, was placed on a slide.[33] The blood was observed for macroscopic clumping and rouleaux formation, which was not obvious. One drop of saline was added to the blood and the blood was evaluated both macroscopically and microscopically for the presence of red blood cell clumping or rouleaux formation. Under the microscope, intermittent, mild rouleaux formation remained but significant rouleaux formation or clumping of the red blood cells was not seen. The absence of red blood cell clumping and significant rouleaux formation after the addition of saline was a nonspecific finding and did not rule out an autoimmune process. However, the normal packed cell volume was not consistent with anemia and, combined with the dispersement of red blood cell clumps and lack of blood parasites and spherocytosis on the blood smear, indicated that autoimmune hemolysis or hematological parasites were less likely as the cause of the icterus and bilirubinuria. Additional diagnostics, such as a pathology review of the complete blood count by a board-certified veterinary pathologist or a Coombs' test, were not elected based on the findings of the complete blood count, blood smear, and saline agglutination test but may have been elected if

the screening tests were ambiguous.

The chemistry profile, which showed significant liver enzyme and TBIL elevations, indicated that either hepatic or posthepatic hyperbilirubinemia was probable.[31] The significant elevations of both ALKP and GGT indicated that biliary disease or obstruction and cholestasis were probable.[31,34,43,45] Primary bone disease or kidney disease were considered unlikely as the renal enzymes were within the reference range and no signs of lameness or pain were found on physical examination. An intestinal abnormality as a cause of the elevated ALKP could not be ruled out but also seemed unlikely as consistent gastrointestinal signs were not reported.

Significant hepatocellular damage was represented by the elevations of both ALT and AST.[45,46] As with bone disease, no signs of muscle disease were found on physical examination and therefore was considered an unlikely cause of AST elevation, while hemolysis did not appear to be present. The mild hypochloremia was most likely the result of decreased chloride intake as no vomiting had been observed, the renal enzymes and urinalysis were unremarkable, and gastrointestinal stasis or obstruction, although not definitively ruled out, was considered less likely based on the radiographic appearance of the stomach and small intestine.[103] Diabetes mellitus and nephrotic syndrome could be eliminated as the renal enzymes, urinalysis and blood glucose were not consistent with these conditions.

Significant bilirubinuria without evidence of hemolysis was confirmed on the in-house urinalysis. This finding was another indication of either hepatic or posthepatic cholestasis.[9] The absence of urobilinogen in the urine also implied that a posthepatic biliary obstruction was present but was interpreted with caution.[9] The trace proteinuria was not considered to be

clinically significant.[52] Significant proteinuria or glucosuria was not identified, making protein losing nephropathy, nephrotic syndrome and diabetes mellitus less likely as causes of the weight loss. The urine specific gravity was consistent with adequate concentration and was not consistent with renal disease or polydipsia. The absence of parasite ova in the fecal sample implied that parasitism was not the likely cause of anorexia, gastrointestinal signs, or weight loss.

The abdominal radiographs, which revealed an enlarged liver and loss of detail in the right cranial abdominal quadrant, also indicated that hepatobiliary disease or pancreatic disease were the primary differential diagnoses of the clinical signs.[54,106] The caudodorsal shift of the gastric axis, an imaginary line running between the fundus, body, and pylorus of the stomach, was consistent with enlargement of the liver, whereas a normal gastric axis will run parallel to the ribs or perpendicular to the spine.[54] The unstructured interstitial pattern in the caudal lungs was most attributable to an artifact or underexposure.[105] Other causes of the interstitial pattern, such as pneumonitis or an inhalant or toxic insult, were considered less likely as previous respiratory abnormalities were not reported and normal inspiratory and expiratory movement of air in the lungs was present on thoracic auscultation.[105] Three-view radiographic views would have been beneficial to allow better assessment of the abdomen, while three-view thoracic radiographs should have been considered to fully evaluate the lungs as well as other thoracic structures.

Infectious hepatic disease, primarily leptospirosis due to its zoonotic potential, or a hepatotoxic insult were primary concerns at the beginning of treatment. Even though the dog was afebrile

with no signs of renal disease, leptospirosis could not be immediately ruled out. Precautions were instituted to minimize the potential of exposure of those tending to the dog and to other animals in the hospital.[39] The administration of ampicillin as part of the initial prophylactic antibiotic regimen was elected to treat potential leptospirosis, as well as provide coverage against anaerobes and gram-negative aerobes in other hepatobiliary disease.[1,39,53] A sample of blood was collected prior to the start of antibiotics in case a leptospirosis PCR was needed for definitive diagnosis.

In addition to ampicillin, the initial treatment focused on providing IV fluids at a rate that allowed correction of the estimated dehydration while providing daily fluid requirements, treatment of potential infectious disease, pancreatic disease or ascending gastrointestinal infection, and alleviating pain through the use of opioids. The daily fluid requirement of the dog was calculated to be 220 ml/day, or 9 ml/hr.[86] The correction of 7% dehydration would require 350 ml/24 hours, or 14 ml/hr.[86] Therefore, a 5 ml/hr rate of the balanced electrolyte solution was administered. Ongoing losses were not anticipated as no vomiting or diarrhea was reported or noted in the hospital. However, the correction of 7% dehydration, as opposed to 5% dehydration, would allow fluid compensation for unanticipated fluid loss through vomiting or diarrhea if they occurred. Additionally, correction of fluid and potential electrolyte abnormalities should be accomplished prior to any surgery if possible.[53]

Additional antibiotics, enrofloxacin and metronidazole, were chosen based on effective combined coverage of aerobic, gram-negative and gram-positive, and anaerobic bacteria.[1,53,84] A low dose of hydromorphone was selected for pain control based on the perceived moderate pain score. The lower dose of hydromorphone was selected as it is metabolized in the liver.[79]

Although vomiting had not been seen, maropitant was administered for the prophylactic treatment of nausea, potential treatment of visceral pain, and reduction of adverse effects seen with the use of opioids.[64,75,76] Famotidine was continued as an injectable antacid as it was the only injectable antacid available.

The use of antibiotics at this point in treatment was controversial. There was no distinct indication of infection, only mild inflammation as indicated by the mild monocytosis, on the complete blood count.[102] A definitive diagnosis had not yet been achieved, and the use of antibiotics prior to a diagnosis may reduce positive culture results in cases where hepatobiliary infection is present.[41,82] However, as stated earlier, there was initial concern for leptospirosis due to its zoonotic potential. The choice to administer ampicillin was suitable as treatment for leptospirosis should not be delayed pending the results of diagnostic testing.[39] The significantly elevated ALT, as well as the elevation in AST, indicated that hepatocellular injury or inflammation existed in the dog.[45,46] Infectious hepatitis, possibly caused by Clostridium piliformis or Escherichia coli, as well as a GBM or other potentially infectious gallbladder diseases, were considered viable differential diagnoses when antibiotics were started. The elected dose of metronidazole was in the upper range of the recommended 7.5-10 mg/kg dose for patients with hepatic disease.[84] Although this dose falls within the listed doses in the literature and may have allowed better anaerobic bacteria coverage, the typical 7.5 mg/kg dose of metronidazole in cases of hepatic disease was likely more appropriate since the functionality of the liver was unknown. Additionally, better gram-positive aerobic coverage, such as with the use of cefazolin, should have been considered in the initial antibiotic therapy.[1]

On the second day of hospitalization, the degree of icterus had visibly worsened. An abdominal

ultrasound was elected to further differentiate between the suspected disease processes; on ultrasound, the gallbladder was subjectively over distended with a striated appearance of the bile, both of which are characteristics of mucocele formation without the classic "kiwi" or stellate pattern within the gallbladder lumen.[4,56] The distended size, and suspected reduced contractility, of the gallbladder was confirmed when the gallbladder volume was estimated, which was significantly greater than 1 ml/kg of body weight.[5] Alone, the length and width of the gallbladder, which were approximately 4 cm and 2.5 cm, respectively, were slightly greater than 1 ml/kg of volume without considering the height of the gallbladder.[5] In order to see if the gallbladder debris was mobile or gravity-dependent, the gallbladder was reassessed in a standing position. The striated appearance of the debris did not change, indicating that the intraluminal contents of the gallbladder were immobile. The dilated and slightly tortuous cystic duct was a final clue that the gallbladder distension was secondary to obstruction rather than anorexia.[58] The normal, non-thickened walls of the gallbladder were not obviously consistent with acute or chronic cholecystitis although it could not be ruled out.[58]

The dilated and mildly tortuous common bile duct to the level of the duodenal papilla and level of hyperbilirubinemia was suggestive of an extrahepatic common bile duct obstruction without the presence of the mucocele. The common bile duct was dilated to 0.5 cm in width, making it easy to identify, whereas the common bile duct may be difficult to identify in normal dogs.[58] Since the normal width of the common bile duct is reported to be less than 0.3 cm, the degree of dilation was considered to be moderate.[58] Further evidence of bile duct obstruction was revealed in the presence of echogenic debris or mucus with anechoic fluid moving back and forth in the dilated bile duct synchronous with breathing. This finding implied that bile could

not exit the bile duct. Aside from mucus in the bile duct, other causes of extrahepatic biliary obstruction, such as a cholelith, gastrointestinal inflammation or neoplasia, were not seen. The degree of focal pancreatitis did not appear to be severe enough to overtly cause posthepatic common bile duct obstruction as the localized inflammation did not involve the common bile duct or duodenal papilla.

The discomfort noted when applying pressure to the area of the right cranial abdomen was consistent with a positive sonographic Murphy sign.[58] A consistent reaction indicating pain, including movement and an increased respiration rate, was seen when pressure was specifically applied to the gallbladder. Signs of pain were not seen when pressure was applied to the focal area of pancreatitis. The adrenal gland widths fell within the upper limit of normal adrenal gland width in small dogs without hyperadrenocorticism.[19]

Since a classic mucocele was not present and a definitive cause of common bile duct obstruction was not evident, the decision to pursue surgery in this case, as opposed to another 24-48 hours of conservative treatment, close monitoring with ultrasound for signs of increased common bile duct dilation or signs of impending gallbladder rupture, or monitoring of biochemistry profiles, was debatable. Gallbladder aspiration for culture or sensitivity was not considered due to the potential risks involved with the procedure, including bile leakage, bradycardia due to vagal stimulation, bacteremia, and local hemorrhage.[5,11] A partial obstruction, or a potential resolving obstruction, could not be completely ruled out. Referral to a board-certified radiologist for additional imaging to further assess for a partial or resolving obstruction was discussed but was not a viable option for the owner. Certainly surgery, especially biliary surgery, poses significant risks to the patient as indicated by the 20-40%

perioperative mortality rate reported in the literature for cholecystectomy.[41] However, the ultrasound findings showed that the debris within the gallbladder was non-mobile and was suggestive of a GBM without the classic "kiwi fruit" appearance.[4] Studies have shown that the ultrasonographic pattern of a GBM may not be a valid basis for treatment recommendations in dogs.[4] In other words, a GBM that lacks the classic "kiwi fruit" appearance is no more likely to respond to medical treatment than a classic-appearing GBM.[4] Additionally, the positive sonographic Murphy sign in the area of the gallbladder, in combination with elevated liver enzymes, hyperbilirubinemia, hyperbilirubinuria, and developing to worsening icterus, strongly suggested that exploratory laparotomy with possible cholecystectomy and common bile duct lavage was needed. There was also concern that chronic cholestasis may result in irreparable hepatic injury, dilation of the bile duct, proliferation of ductules, and fibrosis.[5,9]

Prior to surgery, the dog had been adequately stabilized with correction of dehydration over the previous day of hospitalization. The increase in the hydromorphone dose seemed to be adequate for appropriate analgesia. Although the risk of adverse effects with the higher dose of hydromorphone was greater due to possible decreased hepatic metabolism, no ill effects from the higher dose were noted.[79] The lower dose of hydromorphone (0.1 mg/kg) as a premedication was selected because it was being used for pain control and had been administered within two hours of surgery. Mild sedation from the previous administration of hydromorphone was already present. A lower premedication dose of hydromorphone was also considered due to hepatic disease.[79] The use of benzodiazepines, in combination with hydromorphone, may have been used but the degree of sedation with the use of hydromorphone alone was adequate.[88] Maropitant had been administered prior to sedation to

alleviate potential nausea or vomiting caused by hydromorphone and to treat potential visceral pain associated with the surgery.[75,76]

A major deficiency in the management of this case was the failure to assess coagulation times or administer vitamin K prior to surgery. Coagulation abnormalities are possible in dogs with hepatobiliary disease since the liver synthesizes all coagulation factors, except for factor VIII, and is also the site of activation of vitamin K-dependent clotting factors.[25] No outward signs of abnormal coagulation, such as bleeding at venipuncture sites, were observed and the majority of vitamin K factors must be depleted before coagulation abnormalities are seen.[25] However, real challenges to hemostasis, as with the surgery itself or hepatic biopsy, may have led to hemorrhage if clotting times were abnormal.[25] An in-house coagulation analyzer was not available. In light of this, a mucosal bleeding time should have been performed or vitamin K should have been administered for at least 24 hours prior to surgery.

Propofol was used for anesthetic induction in this case as it is recommended in patients with liver disease and is short acting.[88,89] Increased recovery time due to decreased metabolism of the drug in the diseased liver was considered and monitored following surgery. Isoflurane was the only anesthetic maintenance gas that was available but is recommended for use in patients with hepatic disease.[88]

The surgical technique used to remove the gallbladder allowed adequate visualization of the anatomy to guide resection, assessment of the surrounding tissue for evidence of focal bile peritonitis, and suitable hemostasis once the cystic artery was ligated. During surgery, the common bile duct was deemed to be patent by manual expression of bile without further

dilation of the common bile duct. The right middle liver lobe was selected for a biopsy as it was easily visible and located adjacent to the gallbladder. After prolonged placement of hemostats failed to provide satisfactory hemostasis when acquiring the liver biopsy, guillotine sutures were placed around the margins of the biopsy site which stopped any residual bleeding. No other biopsies were obtained during surgery as the rest of the abdomen was normal in appearance. The pancreas was grossly normal with no outward signs of pathology, such as pancreatitis or neoplasia, when visually inspected. Combined with minimal parenchymal change within the pancreas on ultrasound and lack of involvement with the duodenal papilla, the risk of a pancreatic biopsy was not considered to be justified.

During surgery, the common bile duct was deemed patent through normal manual expression. Common bile duct flushing is generally recommended in cases of a gallbladder mucocele and cholecystectomy but may not always be needed if the common bile duct is deemed patent.[53] Flushing of the common bile duct via the duodenal papilla should have been done to ensure its patency after mucous was noted in the common bile duct during the ultrasound.

Although hypotension under anesthesia is a frequent occurrence, the current fluid rate and previously administered fluid quantity was considered to be adequate for countering potential hypotension while preventing possible fluid overload with higher fluid rates.[87] MAP under anesthesia remained within normal parameters. The dose and frequency of hydromorphone were increased when the dog still appeared to be in discomfort following the initial administration of the opioid. This dose and frequency allowed good pain control both prior to and after surgery. The removal of the source of discomfort, the gallbladder, also reduced pain. A multimodal approach to pain medication may have been considered but the use of

hydromorphone alone appeared to provide adequate analgesia in the postoperative period.

Cefazolin was administered at induction to provide additional antibiotic coverage against gram positive bacteria in addition to antibiotic coverage already provided by the current use of ampicillin, enrofloxacin and metronidazole.[1] This was primarily done due to the probability of performing a cholecystectomy and the possibility of gallbladder rupture or unknown bile peritonitis. The combination of antibiotics administered before, during, and after surgery was chosen to provide broad spectrum coverage against commonly implicated infectious agents in hepatobiliary disease.[1] This was important because, although positive culture results from the gallbladder or liver in cases of a gallbladder mucocele were highly variable in the literature and the actual risk of infection may be low, an infectious process could not be ruled out. The use of antibiotics also limited the potential of an iatrogenic infection during and after surgery. Omeprazole, as opposed to famotidine, was prescribed as an antacid to treat possible gastritis or gastroenteritis secondary to the GBM, surgery, or the oral antibiotics or pain medications. Omeprazole was chosen as proton pump inhibitors have been shown to be superior to H2-receptor blockers in increasing gastric pH.[73]

MAP was continually measured in the immediate postoperative period but was only measured once in the 24 hours following surgery. Hypotension was not noted in the immediate postoperative period or on the single measurement the day after surgery. Lactate was not monitored prior to or, more importantly, after surgery. As mentioned in the literature, post-operative hypotension, usually within the initial 12 hours after surgery, was highly associated with poor clinical outcome and may be secondary to systemic inflammatory response syndrome or sepsis.[41] More frequent or consistent monitoring of systemic blood pressure and the

measurement of lactate, such as every two or three hours in the initial 12 hours after surgery, would have allowed early detection and appropriate management if hypotension was present.

Once the GBM was diagnosed and surgery was performed, and in combination with the lack of renal involvement or other clinical signs associated with leptospirosis, ampicillin was discontinued in favor of a cephalosporin since leptospirosis was considered less likely. Precautions with handling the patient were still maintained. Submission of the leptospirosis PCR was not elected due to the diagnosis of the GBM and lack of clinical signs but was needed to definitively rule out the disease. The use of antibiotics for four weeks after surgery, as opposed to only two weeks of therapy, allowed an adequate time frame to resolve any infectious agents that were present. Moreover, the four week follow-up allowed time for the anticipated resolution of the elevated liver enzymes prior to the scheduled recheck.[44]

The post-operative use of meloxicam as an anti-inflammatory analgesic was controversial. Although the elevated liver enzymes were secondary to the GBM and subsequent cholestasis, hepatic dysfunction, specifically decreased glucuronidation, could not be ruled out at the time of use. It was likely that the dog was more at risk for gastrointestinal ulceration with concurrent liver disease. Oral opioids or a NDMA receptor antagonist medication combined with Tramadol would likely be considered a safer choice for pain control.[77,88]

The gallbladder and liver biopsy obtained during surgery was pertinent to determine if the pathology within the liver was secondary to the mucocele or other underlying hepatic disease. A GBM and cholecystitis were confirmed on the biopsy. Histopathologic analysis of the liver tissue determined that the changes noted within the liver, namely vacuolar hepatopathy and

biliary hyperplasia, were largely secondary to the mucocele and subsequent cholestasis. In cases of a GBM, the histopathologic changes in the liver resolve. Copper staining, which provides a qualitative estimate of copper accumulation, of the liver tissue did demonstrate moderate amounts of centrolobular copper accumulation in zones 2 and 3 within the liver.

In this case, the primary causes of copper accumulation within liver tissue, which is often seen in areas of vacuolar degeneration, may include chronic cholestasis and/or primary hereditary copper toxicosis.[37] Hepatic copper concentrations in dogs with secondary copper accumulation generally fall in zone 1 of the liver while primary copper accumulations begin in zone 3 of the liver.[38] Although neither Yorkshire Terriers nor Miniature Schnauzers are breeds that are associated with copper storage hepatopathies and cholestasis would seem most likely as the cause of hepatic copper accumulation, the presence of copper in the centrolobular areas of the liver suggested that a primary copper hepatopathy may also be present. Copper quantification to further assess the amount of copper in the liver tissue was discussed with the owner but declined. The use of a d-penicillamine to remove the copper from the hepatic tissue was also discussed but the owner favored monitoring of the liver enzymes for further signs of hepatitis.

Culture and sensitivity of the liver biopsy and the bile within the mucocele was not elected for several reasons. The use of antibiotics without a culture and sensitivity, which was considered vital at the time the underlying condition was identified and the need for surgery was realized, could potentially result in a false negative, negating the benefit of the test. The actual percentage of positive growth on cultures in cases of a gallbladder mucocele is low according to the literature, a fact that is likely influenced by the use of antibiotics prior to culture.[41] The antibiotic combination used during and after surgery most likely provided adequate coverage

against bacteria commonly implicated in biliary disease, including gram-positive and gram-negative aerobes and anaerobes.[1,53] Regardless, culture and sensitivity of both the liver and bile should have been pursued to definitively assess for infectious agents that may not have been susceptible to the chosen antibiotics.

On the initial chemistry panel following surgery, the normalization of the ALT, AST, GGT and TBIL indicated that hepatocellular damage and cholestasis had resolved following the cholecystectomy. However, a continued elevation of ALKP, along with elevations of both serum cholesterol and triglycerides, was present. The half-life of ALKP, or the time it takes for ALKP to be reduced to half its original level, is approximately 70 hours, or three days, in normal circumstances.[44] This indicated that ALKP levels should have decreased to normal limits in the month since the cholecystectomy and that an abnormality was continuing to cause release of ALKP from the biliary brush border or epithelial cells.[43] The exact cause of the persistent ALKP elevation after surgery was unknown but could have been due to several reasons. Continued vacuolar hepatopathy or degeneration secondary to the mucocele was considered a likely cause. Other possible causes of elevated ALKP following surgery may include some degree of hepatic fibrosis or scarring, sub-clinical intrahepatic cholestasis, or hyperlipidemia.[43] Pituitary-dependent hyperadrenocorticism could not be ruled out as a cause of the elevated ALKP or the hyperlipidemia. However, since no clinical signs of hyperadrenocorticism, such as polyuria, polydipsia or polyphagia, were present in combination with normal adrenal gland measurements on the initial ultrasound and moderately concentrated urine on the repeated urinalysis, hyperadrenocorticism was considered an unlikely cause of the elevated ALKP and triglyceride levels or contributing disease to GBM formation.[19,21] The effect of a potential

primary copper hepatopathy was questionable since signs of hepatocellular injury or hepatitis, indicated by elevations in ALT or AST, had resolved. Overall, the results of the initial chemistry panel after surgery showed significant improvement in the majority of the hepatic analyte abnormalities that were present prior to the cholecystectomy.

The elevation in the serum triglyceride levels was severe. A documented fast prior to the chemistry panel could not be definitively confirmed. Since hyperadrenocorticism was considered unlikely but could not be ruled out, the primary considerations for the hyperlipidemia, specifically the severe hypertriglyceridemia, were a non-fasted sample or a genetic or breed-associated primary hyperlipidemia.[3] The diet was changed from the bland diet, which contained 23 g/Mcal of fat, to a lower fat diet, which contained 19 g/Mcal of fat, with a recheck of the liver values and serum lipid levels in 8 weeks.[24] The lower fat content in the new diet may also be of benefit if residual or minor pancreatitis was present.

Because of the improved but elevated ALKP, SAMe was continued due to its potential antioxidant properties and because it is a precursor to glutathione, an important component of metabolic processes and cell detoxification within the liver.[67] Due to potential intrahepatic cholestasis as a cause of the continued ALKP elevation, ursodeoxycholic acid was prescribed. The benefits of using ursodeoxycholic acid in the dog were weighed against potential complications associated with biliary obstruction, although the prokinetic effect of ursodeoxycholic acid is debatable.[66] The medication was used to decrease intestinal absorption of cholesterol and suppresses hepatic synthesis and secretion of cholesterol, thereby reducing cholesterol saturation in the bile and increasing bile flow.[66] Ursodeoxycholic acid may also reduce inflammatory changes and fibrosis in the liver.[66] Although continued biliary obstruction

could not be ruled out, the use of ursodeoxycholic acid seemed safe as the TBIL was within normal parameters.

Improved but persistent ALKP elevation was present on the 90-day post chemistry panel after a confirmed fast. The fasted serum cholesterol levels dropped slightly from the 30-day panel but remained elevated, while the serum triglyceride increased significantly. Mild bilirubinuria, which is considered a normal finding in dogs, was present on the urinalysis.[52] Differential diagnoses for the continued elevation of ALKP remained unchanged. The total thyroxine in the bloodwork was 1.2 ug/dL (0.8-3.5 ug/dL), a normal but decreased value within the reference range. The low but normal value indicated that hypothyroidism was not a likely cause of the hyperlipidemia or the GBM, while nonthyroidal illness, such as hepatic disease, may have possibly contributed to the decreased but normal thyroxine level.[23] Measurement of free thyroxine and thyroid-stimulating hormone levels would have provided more information on the overall functionality of the thyroid gland.

The purpose of the repeat abdominal ultrasound was to assess the liver for potential changes associated with causes of consistent ALKP elevation and evaluate the adrenal glands for changes in size or structure. Although nonspecific, the ultrasound findings were suggestive of possible mild hepatic fibrosis, hepatic remodeling, and vacuolar hepatopathy around the cholecystectomy site, as indicated by the increased hepatic echogenicity and mildly increased echogenicity of the portal vasculature in the mid to right liver.[58] The focal area of pancreatitis appeared to have resolved, decreasing the possibility of persistent pancreatitis as a cause of the hyperlipidemia or contributor to the elevated ALKP.[59] Chronic pancreatitis could not be ruled out.[59] The bilateral adrenal glands were not considered to be obviously consistent with pituitary

dependent hyperadrenocorticism as their width remained unchanged from the initial ultrasound and within the upper limit of the cut off measurement in small dogs without hyperadrenocorticism.[19]

In addition to the structural assessment of the liver with ultrasound, preprandial and postprandial bile acids were elected to assess liver functionality. The test involved submitting two blood samples, a fasted sample and sample drawn two hours after a small meal. The bile acids were normal and indicative of normal hepatic function, eliminating hepatic insufficiency as a cause of continued hypertriglyceridemia.

Despite the lack of clinical signs and normal appearance of the adrenal glands on ultrasound, a LDDST was performed to definitively assess for hyperadrenocorticism as a cause of the elevated ALKP and serum lipid elevations. This test was chosen as a screening test for pituitary-dependent hyperadrenocorticism since it has a higher sensitivity and specificity compared to other screening tests.[18] The LDDST was performed by drawing an initial sample of blood followed by the administration of 0.01 mg/kg (0.53 mg) dexamethasone sodium phosphate IV. Additional blood samples were drawn at four and eight hours following the administration of the dexamethasone sodium phosphate. The initial cortisol sample was elevated and attributed to increased cortisol release due to anxiety or stress.[108] As no screening test for pituitary dependent hyperadrenocorticism is 100% accurate, the test showed suppression of serum cortisol levels in both the four and eight hour samples and confirmed that hyperadrenocorticism was not a likely factor in the development of the mucocele, the ongoing elevation of ALKP, or hyperlipidemia.[18]

Additional diagnostics to investigate the continued ALKP elevation and hyperlipidemia may have included a sex hormone panel following an adrenocorticotropic hormone stimulation test to assess for atypical hyperadrenocorticism.[18] However, clinical signs of hyperadrenocorticism were not evident, so the justification of running this test was dubious.[18] A Specific Canine Pancreatic Lipase Immunoreactivity test to assess for chronic pancreatitis may have also been considered. However, since the SNAP cPL was normal, it was likely that the Specific Canine Pancreatic Lipase Immunoreactivity test would also be normal since the diagnostic methodology of the tests is the same.[49]

Following the severe elevation of serum triglycerides in a fasted blood sample and the designation of potential causes of a hyperlipidemia as either not present or unlikely, a primary hyperlipidemia was highly probable. Additionally, primary hyperlipidemia, specifically hypertriglyceridemia, is reported in Miniature Schnauzers, a breed in which mucoceles are known to occur more frequently.[3] The dog was a Yorkshire Terrier and Miniature Schnauzer mix breed so an inherited predisposition to hyperlipidemia was suspected. The likely cause of the disorder was high blood concentrations of chylomicrons and VLDL since the triglyceride levels were severely elevated.[24] Additional testing, such as the serum turbidity test and the refrigeration test, may have been considered to further clarify the type of hyperlipidemia.[24] The change to a diet with decreased fat did not seem to affect the hypertriglyceridemia. The severe triglyceride elevation warranted aggressive medical treatment with pharmaceutical lipid-lowering agents.[24,27] Due to potential side effect with lipid-lowering medications, the owner elected to supplement fish oil and chitosan with continued monitoring of the triglyceride levels.[27]

The reason for the development of the GBM in the dog is unknown but the primary hyperlipidemia was highly suspected to have played a role in the development of the GBM. The chronic hyperlipidemia may have led to decreased gallbladder motility, resulting in prolonged exposure of the gallbladder mucosa to concentrated cytotoxic, hydrophobic bile acids.[3] The development of biliary sludge secondary to the hyperlipidemia may have also played a role, further decreasing gallbladder motility.

Summary

A dog initially presented for perceived gastroenteritis or possible pancreatitis secondary to dietary indiscretion. Conservative treatment failed to resolve the symptoms while icterus and abdominal discomfort developed. Significant biochemical abnormalities included elevated liver enzymes and evidence of cholestasis. An atypical gallbladder mucocele and posthepatic biliary obstruction were diagnosed with ultrasound. A cholecystectomy was performed followed by a normal postoperative recovery. Histopathology confirmed the presence of the mucocele and typical changes in the liver associated with chronic cholestasis. Consistent postoperative elevations of ALKP were suggestive of continued or long lasting adverse effects of the hepatic parenchyma due to the mucocele or reactive hepatopathy secondary to a suspected genetic or breed- associated hyperlipidemia. The long term prognosis was considered to be good since liver functionality was normal. However, continued monitoring of the liver enzymes and treatment of the persistent hypertriglyceridemia are necessary.

Endnotes

a. SNAP cPL test, IDEXX Laboratories, Norcross GA

b. Lactated Ringer's Solution, Abbott Laboratories, North Chicago IL

c. Famotidine 10 mg/ml, West-Ward Pharmaceuticals, Eatontown, NJ

d. Cerenia 24 mg, Zoetis Inc, Kalamazoo, MI

e. Science Diet i/d, Hills, Topeka, KS

f. petMAP graphic, Ramsey Medical Inc, Tampa, FL

g. HemaTrue Hematology Analyzer, Heska Corp, Des Moines, IA

h. DRI CHEM 7000 Chemistry Analyzer, Heska Corp, Des Moines, IA

i. Vet-10 urine reagent strips, Jorgensen Laboratories INC, Loveland, CO

j. Fecal loop, Innovative Veterinary Products, New Buffalo, MI

k. Fecasol, Vetoquinol USA, Ft. Worth, TX

l. Innovet Classic, Summit, Chicago, IL / Revo Duoview DR, Kennesaw, GA

m. 18-gauge intravenous catheter, Covidien Animal Health, Mansfield, MA

n. Ampicillin sodium 200 mg/ml (reconstituted), Sandoz Inc, Kundl, Austria

o. Metronidazole 5 mg/ml injectable, Hospira Inc, Lake Forest, IL

p. Baytril 22.7 mg/ml injectable, Bayer Animal Health, Shawnee Mission, KS

q. Mini-infuser 300XL syringe pump, Baxter Medical, Deerfield, IL

r. Hydromorphone 2 mg/ml, West-Ward Pharmaceuticals, Eatontown, NJ

s. General Electric Logiq e Vet, Sound Technologies, Carlsbad, CA

t. Propofol, Abbott Laboratories, North Chicago, IL

u. Isoflurane, Piramal Enterprises, India

v. Vetroson Oxy-gen System, Summit Hill Labs, Trenton Falls, NJ

w. BM7 Vet, Bionet America Inc, Tustin, CA

x. Cefazolin, West-Ward Pharmaceuticals, Eatontown, NJ

y. Betadine, MWI Animal Health, Boise, ID

z. Alcohol, Sam's Club, Bentonville, AR

aa. Metzenbaum scissors, Integra Miltex, York, PA

bb. Kelly hemostat forceps, Integra Miltex, York, PA

cc. 2-0 polydioxanone, PDS II, Ethicon, Sommerville, NJ

dd. 3-0 polydioxanone, PDS II, Ethicon, Sommerville, NJ

ee. Cefpodoxime 100 mg tablets, Cronus Pharma LLC, East Brunswick, NJ

ff. Metronidazole 50 mg tablets, Roadrunner Pharmacy Corp, Phoenix, AZ

gg. Enrofloxacin 22.7 mg, Putney Inc, Portland, ME

hh. Metacam 0.5 mg/ml, Boehringer Ingelheim, St. Joseph, MO

ii. Tramadol 50 mg, Sun Pharmaceuticals, Cranberry, NJ

jj. Omeprazole 10 mg, Apotex Corp, Weston, FL

kk. Denamarin, Nutramax Laboratories, Lancaster, SC

ll. Antech Diagnostics, Sandy Springs, GA

mm. Ursodiol 25 mg/ml, Roadrunner Pharmacy Corp, Phoenix, AZ

nn. Gastrointestinal Low Fat, Royal Canin USA, St. Charles, MO

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