Natural History of Biliary Sludge in Dogs

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ABSTRACT

Background: Biliary sludge is associated with gallbladder (GB) dysmotility and mucus hypersecretion suggesting that these factors could lead to GB mucoceles. If biliary sludge does progress to GB mucoceles, treatments to reduce the production and progression of sludge are warranted.

Objectives: The aim of this study was to determine the natural history of biliary sludge in dogs.

Animals: Healthy, client-owned dogs (n=74) screened for biliary sludge; 42 affected dogs identified

Methods: Prospective, observational design. Serial ultrasound examinations and biochemistries were evaluated over 1 year. The following were determined: percentage of the GB filled with sludge (mild (0.01%-24.4%), moderate (24.5%-49.4%), moderate to severe (49.5%-74.4%), severe (74.5%-100%)), gravity dependency of sludge, GB dimensions, and biochemical indices (ALT, GGT, ALP, total bilirubin, albumin, total calcium, triglycerides, and cholesterol). Mixed model ANOVA, Friedman chi-square, Mantel-Haenzsel chi-square tests, and Kruskal-Wallis test were performed to detect significant changes in these parameters. Significance at $P < 0.05$.

Results: After 1 year of follow-up, the percentage of the GB filled by sludge was mild (34%), moderate (47%), moderate to severe (13%), severe (3%), or absent (3%) with no significant difference in the median degree of biliary sludge within 1 year ($P=0.36$). There was no significant change in the gravity dependency of sludge over 1 year. Dogs had resolved (2%), decreased (19%), static (40%), increased (29%), or recurrent (10%) sludge at the conclusion of the study. Biochemical indices or GB volume were not significantly different over time or among groups.

Conclusion: Biliary sludge is prevalent, affected dogs remain asymptomatic, and it rarely resolves in healthy dogs over a period of 1 year. Some dogs developed non-gravity dependent sludge within 1 year, which may indicate changes in consistency.

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ABBREVIATIONS

ALP - alkaline phosphatase

ALT - alanine aminotransferase

BD -Bile Duct

cAMP - cyclic adenosine monophosphate

CCK - cholecystokinin

CMH - cystic mucinous hyperplasia

EHBO - extrahepatic biliary obstruction

ERCP - endoscopic retrograde cholangioportography

GB - Gallbladder

GBM - gallbladder mucocele

GBV - gallbladder volume

GBV/kg - gallbladder volume per kilogram bodyweight

GGT - gamma-glutamyl transferase

PUC - percutaneous ultrasound guided cystocentesis

SOD - Sphincter of Oddi

UDCA - ursodeoxycholic acid

CHAPTER I: Literature Review

A. Gallbladder Structure and Function

a. Anatomy of the Biliary System

The biliary system is comprised of the bile canaliculi, bile ductules, intralobular ducts, interlobular ducts, hepatic ducts, gallbladder (GB), cystic duct, and bile duct (BD).[1] The GB connects to the BD via the cystic duct, and this location denotes the transition of hepatic ducts to the BD.[1,2,3] The BD opens into the duodenum via the Sphincter of Oddi (SOD). The SOD serves as a one-way valve that allows bile flow into the duodenum, but prevents enteric contents from ascending into the biliary system.[1,3] The BD opens next to the minor pancreatic duct at the major duodenal papilla in the dog. The majority of hepatic bile is diverted into the GB where it is stored. Gallbladder bile is eventually expelled into the duodenum from the GB via the cystic and bile ducts. A small percentage of hepatic bile bypasses the cystic duct and is delivered directly into the duodenum via the BD.[1]

The GB is a pear-shaped organ that lies in a fossa between the right medial and quadrate liver lobes and is attached to the hepatic visceral surface.[1-4] The apical aspect of the GB is called the fundus, the middle portion is called the body, and the tapered portion that is confluent with the cystic duct is called the neck.[2] Histologically, the GB is structurally similar to the intestine. The GB contains layers, which consist of a luminal mucosa with microvilli, lamina propria, muscularis, connective tissue, and serosa.[1,2] The mucosal layer contains mucus glands which secrete mucin to protect the luminal epithelium from cytotoxic substances such as bile acids. Mucin secretion is stimulated by inflammation via cytokines, endotoxins, prostaglandins, and increased hydrophobic bile

acids within bile.[1,5] Parasympathetic and sympathetic innervation of the GB is via the vagus nerve and splanchnic nerves respectively.[1,2,4] The cystic artery, which is the left branch of the hepatic artery, is the sole source of blood supply to the GB.[1,3,4]

b. Function of the Biliary System

The biliary system transports bile from liver to its eventual destination in the duodenal lumen. Bile serves as a source of bile acids for fat digestion and absorption, a source of bicarbonate to buffer hydrogen ions in the duodenal contents, and an elimination route for lipophilic metabolic products and xenobiotics.[1,6] The majority of bile is stored, concentrated, and modified in the GB during the interdigestive phase. During this phase, bile is continuously delivered into the relaxed GB.[1] Gallbladder contractions and delivery of bile to the duodenum occurs in both the interdigestive and postprandial states.

In the fasted state, gastrointestinal contractions occur in response to a periodic motor activity called the migrating motor complex. The GB responds to the migrating motor complex via motilin, which induces "bellows-like" contractions during the interdigestive phase.[1,7] Rhythmic relaxation of the SOD delivers the GB bile into the duodenum in spurts instead of a continuous flow.[1,3,4]

In the postprandial state, GB contraction and secretion is mediated via neurohormonal stimulation to coordinate GB contraction with meal ingestion. Gastric distension, free fatty acids, and amino acids lead to vagal stimulation and cholecystoknin (CCK) release from duodenal enteroendocrine cells (I cells). Cholecystokinin stimulates GB contraction and relaxation of the SOD.[2-4] Cholecystokinin is the most potent stimulus for GB contraction whereas acetylcholine is a weak stimulant. Sphincter of

Oddi relaxation is enhanced by secretin. Acidic chyme entering the duodenum stimulates release of secretin from the S cells in the duodenum and vagal stimulation results in release of vasoactive intestinal polypeptide. Secretin and vasoactive intestinal polypeptide stimulate secretion of mucin and bicarbonate from the GB mucosa. This bicarbonate-rich fluid mixes with the stored bile prior to expulsion into the duodenum.[1] Bile acids are delivered back to the liver via enteroheptic circulation and inhibit further release of CCK.[1,2,4] As fatty acids enter the small intestines, the release of somatostatin and gastrointestinal epithelium inhibits GB contraction.[2] The GB relaxes and SOD tone increases to facilitate the delivery of hepatic bile into the GB. [1,2]

c. Bile Formation and Composition

Bile consists of bile acids, water, electrolytes, cholesterol, phospholipids, proteins, fatty acids, and bilirubin.[1,2,6] Bile components are synthesized and stored within the hepatocytes, and subsequently secreted into the bile canaliculi.[1,2,4,6] Bile flow and formation is driven by osmotic gradients created by the secretion of bile acids and/or other solutes into the bile canaliculi. There are two mechanisms of bile formation and flow, bile-acid dependent and bile acid independent. In bile-acid dependent flow, bile acids are actively transported into the canaliculi. This creates an osmotic stimulus, which draws water and electrolytes into the canaliculi. Bile-acid independent flow is mediated in part by the action of glutathione and secretin. Glutathione creates an osmotic stimulus for bile flow independent of bile acid secretion. Secretin acts on the cholangiocyte to stimulate the release of bicarbonate into the bile canaliculi resulting in a movement of water into the bile duct.[1,6,8,9] Phospholipids, cholesterol, and bile salts form water-soluble mixed micelles. The formation of mixed micelles reduces surface tension and lessens

the osmotic effect of bile acids. This protects the canalicular epithelium from the cytotoxic, hydrophobic bile acids. Bile salts that do not form micelles, such as deoxycholate, have a greater osmotic effect on bile flow. Vagal stimulation, CCK, secretin, glucagon, and cholehepatic shunting of bile acids stimulate hepatic bile flow, whereas release of somatostatin inhibits hepatic bile flow.[1,4] The cholehepatic shunt pathway involves bile acid uptake by the cholangiocyte via sodium-dependent transporters and allows for intrahepatic circulation of bile acids. This pathway is important for signaling cholangiocyte secretion of mucin and bicarbonate. Modification of hepatic bile occurs as bile flows through the ductal system by secretion of bicarbonate, mucin, water, and electrolytes, and reabsorption of water and electrolytes.[1]

Hepatic bile is subsequently delivered into the GB where it is stored, concentrated, modified, and acidified. During storage within the GB, bile is concentrated 10 to 15 fold. The GB has both absorptive and secretory properties mediated by the transport of water and electrolytes across the GB epithelium. Absorption of water leads to the concentration of bile acids, bilirubin, phospholipids, proteins, and cholesterol. In addition to sodium and chloride absorption, the GB also secretes potassium and hydrogen ions.[1,10] Absorption of water and salts are the main functions of the GB epithelium. Intraluminal sodium is required for the transport of water and electrolytes and is the driving force of behind their absorption. Intracellular pH, cyclic adenosine monophosphate (cAMP), and intracellular calcium facilitate the effects of peptide hormones and neurotransmitters on gallbladder mucosal function.[10,11] Prostaglandins, secretin, vasoactive intestinal polypeptide, bradykinin, and vasopressin results in increased intracellular cAMP.[10,11]

The GB secretes fluid abundant in mucin and bicarbonate within the interdigestive phase.[11] Increases in intracellular cAMP and intraluminal bile acids leads to GB mucin secretion. Intraluminal mucin secretion is a protective mechanism of the GB epithelium from the cytotoxicity of bile acids.[10] The hepatic bile is also acidified while stored within the GB. The pH of hepatic bile is 7 to 7.8. Once it enters the GB, bile is acidified to a pH of 6 to 7.[1] Acidification occurs via the absorption of sodium ions in exchange for hydrogen ions. Absorption of bicarbonate may also contribute to the acidity of bile.[10] Secretin acts on the GB epithelium resulting in bicarbonate secretion into the GB lumen.[1,10,11] Prior to expulsion into the duodenum, the concentrated and acidified bile is mixed with bicarbonate-rich fluid.[1]

d. Bile Acids

Bile acids are essential for emulsification and absorption of fats. Bile acids are derived from cholesterol and comprise about 90% of total bile solids. Primary bile acids include cholic acid and chenodeoxycholic acid.[6,12] Prior to secretion, bile acids are conjugated with taurine or glycine.[1,6,12] Dogs mainly conjugate bile acids with taurine but can also utilize glycine. Taurocholate and taurocholic acid are primary bile salt and acid, respectively.[4] Bile acid refers to the unionized form whereas bile salt refers to the ionized form.[13] Conjugation of bile acids lowers the pKa, which favors the ionized form (bile salt) within the bile and duodenum. Bile salts are more soluble than bile acids and, thus, are less likely to be absorbed from the small intestinal lumen. This leads to a higher intraluminal concentration of bile salts for emulsification and absorption of fats.[6,12,13] Water-soluble mixed micelles (bile salts and lipids) aid in fat emulsification and absorption within the intestines. Bile salts also activate lipases and play an important role

in the absorption of fat-soluble vitamins (A, D, E, K). [4] A small portion of primary bile acids that are secreted into the intestines become secondary bile acids, deoxychoilc acid and lithocholic acid, via dehydroxylation by intestinal bacteria.[6,12] The majority of bile acids (95%) are reabsorbed at the ileum via sodium-bile salt cotransporters and subsequently circulate to the liver via portal circulation. This process is known as enterohepatic circulation. Less than 5% of bile acids are lost in the feces per day.[1,4,6]

B. Diagnostic Testing of the Gallbladder

The diagnostic approach to canine biliary disease typically includes assessment of biochemical abnormalities along with abdominal ultrasonography. Abdominal ultrasonography is the basis of hepatobiliary imaging in veterinary medicine. In some cases, nuclear scintigraphy can be used to evaluate the patency of the biliary tract.

In addition to clinical signs, laboratory abnormalities are the first indication of biliary disease. Increased serum alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and alanine aminotransferase (ALT) activities, hyperbilirubinemia and hypercholesterolemia are commonly caused by with biliary disease. Alkaline phosphatase is an inducible liver enzyme that is membrane-associated within canalicular hepatocytes.[1,14] Gamma-glutamyl transferase is also found within membranes of hepatocytes and cholangiocytes. The activities of these two enzymes are commonly increased in cholestatic disorders; however, ALP can also be increased due to hepatic parenchymal disease, induction from drugs, endogenous corticosteroids, or systemic disease.[1,14] Gamma-glutamyl transferase is more specific for biliary disease in dogs but is not as sensitive as ALP. Corticosteroids and anticonvulsants (barbiturates) can also lead to increased serum enzyme activity of GGT in dogs.[14] Increased serum enzyme

activity of ALT can be seen in cholestasis and biliary diseases, but to a lesser magnitude than ALP and GGT.[1,14] This increase in serum ALT enzyme activity is likely a result of cholestasis and inflammation causing hepatocellular injury. Total bilirubin becomes increased in prehepatic, hepatic, or post hepatic disorders. Post hepatic increases in total bilirubin are associated with obstructive biliary disease due to GBM, inspissated bile, cholelithiasis, pancreatitis, and neoplasia. Increased serum cholesterol concentration can occur with obstructive extrahepatic biliary disease.[14]

Abdominal radiography is of limited value in the assessment of biliary disease. Poor serosal detail or a mass-like effect within the right cranial abdominal quadrant can be seen in cases of GB distention due to extrahepatic biliary obstruction (EHBO) pancreatitis, or bile peritonitis.[1,15] Mineralization along the biliary tree and GB wall may be visualized on radiographs in dogs with cholelcystolithiasis or choledocholithiasis, porcelain GB, or dystrophic mineralization. Cholecystoliths, if mineralized, are evident as round opacities within the cranial right ventral aspect of the liver. Choledocholiths within the hepatic ducts are visualized dorsal and caudal to the location of the GB, but are rarely large enough to be seen radiographically.[1,15] Porcelain GB appears as a thin oval radiopaque structure within the right side of the liver and can be seen with long standing cholecystitis, cystic hyperplasia, or neoplasia.[15,16] Emphysematous cholecystitis or cholangitis can be detected on radiographs as gas opacities within the GB, biliary structures, and liver.[15] These findings may also be present with hepatic abscessation. Abdominal ultrasonography is generally needed to further characterize and confirm the suspicion of biliary disorders found on abdominal radiographs.

Cholecystography is a type of radiographic contrast imaging that opacifies the GB and biliary tree for the evaluation of space occupying lesions and biliary obstruction. Various methods have been described such as oral, intravenous, fluoroscopic percutaneous or ultrasound-guided transhepatic cholecystography. This technique has been replaced by ultrasonography. [1,15]

Abdominal ultrasonography is currently the diagnostic tool of choice in evaluating GB and biliary disease. Ultrasonography provides assessment of gallbladder volume (GBV), structure and thickness of the GB wall, intraluminal GB contents, and size and contents of the BD and intrahepatic ductal system. The normal GB appears as an ovoid structure with anechoic contents surrounded by hepatic parenchyma.[17] The GB wall is typically indistinct from the surrounding hepatic parenchyma. The normal GB wall in dogs should measure less than 2-3mm. The BD is not typically apparent via ultrasound in normal dogs and should be less than 3 mm in diameter if visualized.[1,15,18]

Gallbladder volume can be calculated using various formulae based on ultrasound GB measurements, such as length, width, height, and depth. Various formulae, including the ellipsoid method, were evaluated in dogs and shown to accurately estimate true GBV.[19,20] The ellipsoid method for calculating GBV is more commonly used in clinical research and practice.[21] The ellipsoid method utilizes width (W), depth (D) and length (L). Width and depth are measured using the transverse images and length is measure on the longitudinal images. The ellipsoid formula is 0.53 x W x D X L, and GBV is denoted in milliliters. The GBV is normally less than 1 mL/kg body weight, but may be increased in fasted or anorexic dogs. [1,2,15,21] However, one study showed that 86% of dogs with

normal GB contractility that were fasted for 12 hours had GBV less than or equal to 1 mL/kg bodyweight.[21]

Gallbladder wall thickening is a non-specific finding. Generalized wall thickening is seen in cases of cholecystitis, edema (portal hypertension, hypoproteinemia, anaphylaxis, inflammation), and hepatitis, or due to artifact (peritoneal effusion).[18,22] Focal thickening can be seen with neoplasia (adenomas, adenocarcinoma), cystic mucinous hyperplasia (CMH), and cholecytitis.[1,15,18] With cholecystitis, the GB wall can appear thickened with a hyper- or hypoechoic wall with or without a multilayered appearance.[1,18 23] The GB may also appear distended and contain hyperechoic contents.[23] However, these ultrasound findings are neither sensitive nor specific for cholecystitis. A discontinuous GB wall is indicative of GB wall necrosis and/or rupture.[24,25] The GB wall may also have a trilaminar appearance with GB necrosis.[1] Irregular GB wall with frond like projections may be seen with CMH.[1]

Various intraluminal contents occur with biliary disease. Biliary sludge is characterized by gravity dependent, non-shadowing, echogenic material within the GB.[15,26] Choleliths appear as focal, gravity dependent, echogenic structures with distal shadowing.[17,18] Gallbladder mucoceles (GBM) are visualized as an echogenic stellate pattern or kiwi pattern surrounded by hypoechoic structures and GB distention.[24,27] The intraluminal contents comprising a GBM are characterized as non-gravity dependent, inspissated bile (hyperechoic) surrounded by mucin (hypoechoic).[24,27]

Ultrasonographic evidence of EHBO in dogs includes GB distension, BD larger than 3 mm in diameter, and dilatation of the hepatic and intrahepatic ducts. Resolution of EHBO does not always result in resolution of biliary duct dilatation.[28] With EHBO, the

BD becomes dilated 24-48 hours after obstruction. The extrahepatic ducts become dilated 72 hours after obstruction, and within 4-7 days the intrahepatic ducts become dilated.[15,29]

Functional ultrasonography can be performed to assess GB motility in dogs. Gallbladder motility may be disturbed due to functional or mechanical obstruction. There are different protocols to induce GB motility to assess GB emptying. One protocol includes assessment of GB volume after a 12-hour fast. A meal or prokinetic agent, such as erythromycin (motilin analogue), is administered and the GBV is reassessed at 1 and 2 hours. The GBV should be less than 1 mL/kg or have a greater than 25% reduction in gallbladder volume (ejection fraction).[1,21] If GBV or ejection fraction is abnormal, GB dysmotility is likely present.[21] Another protocol assesses GB emptying after injection of sincalide (CCK analogue). In one study, the percentage of GB emptying within one hour of sincalide was less than 20% in dogs with EHBO and at least 40% in normal dogs or dogs with non-obstructive biliary disease, respectively.[30]

Percutaneous ultrasound-guided cholecystocentesis (PUC) is a diagnostic and therapeutic tool. The procedure involves ultrasound-guided aspiration of GB bile via transhepatic or ventral fundic approach. The GB bile can be submitted for cytology and bacterial culture, which will help assess the presence of cholecystitis. Bile culture has a better diagnostic yield than cultures of liver biopsies in dogs with hepatobiliary disease.[31] Therapeutically, PUC has been utilized to decompress the GB in dogs with EHBO resulting in improvement in clinical signs.[32] Percutaneous ultrasound-guided cholecystocentesis has been shown to be a safe procedure in dogs.[32,33] Potential

complications include bile peritonitis, bacteremia, hemorrhage, hemobilia, vasovagal event, respiratory arrest, bradycardia, and death.[1]

Hepatobiliary scintigraphy is used in veterinary medicine to determine GB motility and patency of biliary tract. Hepatobiliary scintigraphy may be useful in differentiating partial versus complete biliary obstructions in dogs where ultrasound findings are inconclusive.[34,35] Hepatobiliary scintigraphy utilizes intravenous infusion of technetium Tc 99 m iminodiacetic acid derivatives such as mebrofenin or disofenin.[17] Evidence of EHBO includes delayed clearance of the radioactive material, normal to decreased hepatic ejection fraction, inability to visualize the biliary tree, or failure to visualize radioactive material within the intestines.[17,34,35] A partial biliary obstruction is characterized by normal hepatic ejection fraction and delayed clearance of radioactive material.[34,35] The lack of radioactive material in the intestines within 3 hours has been used as the cutoff time for diagnosis of complete EHBO. However, transit of radioactive material can be markedly delayed with partial EHBO. It has been shown that a cutoff time of 24 hours instead of 3 hours improves the specificity of scintigraphy in complete EHBO from 33% to 83%. [35] Hepatobiliary scintigraphy is rarely used in the evaluation of canine biliary disease as it is more invasive and time consuming compared to abdominal ultrasonography.

Endoscopic retrograde cholangiopancreatography (ERCP) is a minimally invasive technique that utilizes endoscopy and fluoroscopy for the evaluation of the biliary and pancreatic ducts. Briefly, a side-view endoscope is used to visualize the major duodenal papilla. The papilla is cannulated with a catheter placed through the working channel of the endoscope. Once the catheter is within the biliary or pancreatic duct, contrast

material is injected into the duct and visualized with fluoroscopy.[36,37] Studies have shown the feasibility and safety of this technique in dogs and cats; however, it has yet to become a diagnostic tool in the clinical setting.[36,38,39] This is likely attributable to high equipment costs, and the time and level of expertise required to perform this procedure successfully. In people, ERCP has been used for diagnosis of biliary and pancreatic disease such as cholangitis, microlithiasis and choledocholithiasis, biliary strictures, papillary stenosis, pancreatitis, and pancreatic neoplasia.[37,40,41] In a group of dogs with chronic gastrointestinal signs, ERCP was used to identify biliary abnormalities such as dilated common bile duct, malformation of the bile duct, major papillary stenosis with biliary outflow disturbance, and intraductal filling defects.[39] In addition to its diagnostic utility, ERCP can be used therapeutically in cases of endoscopic papillary sphincterotomy, extraction of choledocholiths, and biliary stent placement in people.[37,41] In one dog with papillary stenosis, endoscopic sphincterotomy was successfully performed resulting in resolution of the dogs clinical signs.[39] Biliary stent placement has been attempted using ERCP in healthy research dogs as well in two dogs with EHBO. This technique was proved to be technically challenging with successful placement of biliary stents in 4/7 research dogs and one dog with EHBO.[42] Short-term complications of ERCP in people include pancreatitis, infection, hemorrhage, and perforation. Clinical signs of short-term complications were absent in dog and cats undergoing ERCP.[36,38,39] ERCP holds promise as a diagnostic and potentially therapeutic tool in biliary and pancreatic disease in dogs.

C. Disorders of the Canine Gallbladder

    a. Gallbladder Dysmotility

Gallbladder dysmotility or biliary dyskinesia is characterized in people by clinical signs of biliary pain without evidence of cholelithiasis or structural biliary disease.[43-45] It has been hypothesized that biliary dyskinesia is related to gallbladder motor and/or SOD dysfunction.[43,44,45] Gallbladder dysmotility has recently been recognized in dogs.[1,46,47] Three dogs that developed GBM had evidence of GB dysmotility prior to GBM formation.[46] Experimentally, GB dysmotility caused by ligation of the cystic duct led to the development of biliary sludge in dogs.[48] Underlying disease processes that lead to GB dysmotility in dogs are undetermined. Gallbladder mucoceles, cholelithiasis, and biliary sludge have been associated with GB dysmotility.[46,47,48] Gallbladder dysmotility in these disorders is suspected to be related to impaired smooth muscle contractility due to inflammation, endogenous steroid hormones, increased bile cholesterol concentration, and CCK receptor dysfuction.[1,47,49] Additionally, impaired GB motility may result from ineffective expulsion of GB contents due to tenacious and semi-solid mucin (GBM) or biliary obstruction.[1,46,47] Steroid hormones such as progestin, androgens, and glucocorticoids may also be associated with GB dysmotility. [46,50-52] Progesterone and androgens have been shown to inhibit multiple signaling pathways responsible for GB smooth muscle contractilty.[50-52] Hypothyroidism has been associated with deceased bile flow and cholelith formation in people.[53] Delayed bile flow with hypothyroidism may be related to changes in bile composition, decreased hepatic bile secretion, increased tonicity of the SOD, and alterations in cholesterol homeostasis.[53-55] Studies assessing GB motility in dogs with GBM as well as in dogs with hyperadrenocorticism, hypothyroidism, and dyslipidemias are warranted. Documentation of GB dysmotility in dogs is rare. This is likely a reflection of the fact that GB motility studies are uncommonly performed in

clinical veterinary medicine. Typically, dogs present for associated diseases (endocrinopathies, GBM, dyslipidemias) and concurrent GB dysmotility may be suspected. In those patients, therapy is guided towards associated diseases with or without choleretic therapy with ursodeoxycholic acid (UDCA). Functional ultrasonography or cholecystokinin-cholescintigraphy can be used to assess gallbladder volumes and/or ejection fractions to aid in the diagnosis of GB dysmotility.[21,44,47,56] In people there is controversy over the best therapy, but this generally includes cholecystectomy and/or sphincterotomy.[43,44]

b. Biliary Sludge

Biliary sludge is defined as the presence of gravity dependent, non-shadowing, echogenic material within the gallbladder.[15,57,58] In some cases, biliary sludge may have an acoustic shadow without evidence of cholelith formation suggesting mineralization of sludge. Sludge balls, which are round aggregates of mobile biliary sludge, have also been described in dogs.[26] In dogs, biliary sludge is solely an ultrasonographic diagnosis. In people, biliary sludge consists of an aggregation of cholesterol crystals, bilirubin particles, calcium salts, and mucin, and is also termed microlithasis. Direct microscopic evaluation of biliary sludge is the gold standard in people as this allows for detection of microliths that are too small to be detected with ultrasond.[59,60] The microscopic appearance and biochemical composition of biliary sludge and the correlation of bile contents and the ultrasonographic findings have not been performed in dogs. A study in people revealed that ultrasonographic biliary sediment can be due to biliary sludge, suppurative debris, non-shadowing calculi, and abnormal accumulations of mucin.[61] Biliary sludge is a common finding in dogs without clinical signs or significant

abnormalities on physical examination, with a prevalence of 53%. The prevalence of biliary sludge in those dogs was not significantly different compared to the prevalence in sick dogs, 62% with hepatobiliary disease and 48% with other disease.[57] Biliary sludge has been found in 35-48% of dogs irrespective of health status.[62,63] The cause and clinical significance of biliary sludge in dogs is currently unknown. Biliary stasis and modification of bile within the GB promote the formation of biliary sludge in people.[58,60,64] Mucus hypersecretion from GB epithelium is associated with biliary sludge in people[65] and with exposure of the GB epithelium to increased concentrations of hydrophobic bile acids in dogs.[5] It is hypothesized that biliary stasis and biliary sludge lead to further inspissation of bile and accumulation of mucin, forming what is known as a GBM.[1,3,24]

Risk factors for biliary sludge formation in dogs have been poorly defined with conflicting findings from various studies. Beagles, Cocker Spaniels, and Poodles were shown to have a higher prevalence of biliary sludge;[62] although, a smaller study failed to identify any breed with increased prevalence of biliary sludge.[63] Studies are conflicting as to whether age is a factor with the majority of reports suggesting biliary sludge is more prevalent in geriatric dogs.[47,57,62] Biochemical markers of hepatobiliary disease and cholestasis have not been correlated with presence of biliary sludge.[57,62] One study show that dogs with biliary sludge had significantly higher serum cholesterol concentrations, but it was unclear whether this was due to the presence of sludge or concurrent disease and medications.[47] The same study illustrated that dogs with biliary sludge have decreased GB emptying when compared to dogs without biliary sludge.[47] Biliary sludge and pigment gallstones formed within three days in dogs with acute ligation of the cystic

duct supporting biliary stasis as a cause of biliary sludge in dogs.[48] The high concentrations of hydrophobic bile acids and cholesterol within sludge may also lead to biliary stasis. Unconjugated, hydrophobic bile acids impair GB smooth muscle contractility as indicated by impaired stimulation with CCK and acetylcholine. The degree of hydrophobicity of bile acids is associated with increased GB volumes due to the effects on the GB smooth muscle.[66] Excessive cholesterol in bile also impairs smooth muscle contraction.[66]

Whether spontaneous biliary sludge in dogs has increased amounts of unconjugated, hydrophobic bile acids and/or cholesterol and what affect this may have on GB smooth muscle function remains to be determined. It has been shown that steroid hormones lead to GB dysmotility and stasis.[1,50-52] Experimental iatrogenic hyperadrenocorticism did not cause a significant increase in the formation of biliary sludge, but the number of dogs in the study was limited and treatment was of short duration.[67] Results of a similarly designed experiment did show an increase in unconjugated bile acids in dogs with iatrogenic hyperadrenocorticism suggesting hypercortisolemia may indirectly lead to mucin hypersecretion via effects of unconjugated bile acids on the GB epithelium.[68] Dyslipidemias, hypothyroidism, and hyperadrenocorticism have been circumstantially associated with biliary sludge, and biliary sludge is suggested to result from concurrent GB dysmotility.[46]

Unfortunately, there is a lack of a consistent definition for biliary sludge in the veterinary literature. The presence of echogenic, non-shadowing, intraluminal GB contents seen with ultrasound is a consistent feature in the definition of biliary sludge.[24,57,62,67] Gravity dependency and/or mobility are typically included in this

definition.[24,57,67] Biliary sludge has also been defined to include both an ill-defined or clear linear interface between echogenic and anechoic bile.[67,69] In people, biliary sludge typically has a stable, well-demarcated, gravity dependent echo-pattern that slowly gravitates versus an ill-defined or free-floating accumulation of echogenic material.[65] When considering a linear distinction between bile and sludge in dogs, only 6.8% of dogs had a linear distinction between anechoic bile and sludge versus 53% of dogs had either distinct linear appearance or ill defined sludge.[57] Information on the fate of biliary sludge in dogs is limited to anecdotal evidence. The percentage of dogs with biliary sludge that have resolution, persistence, or progression of biliary sludge has not been determined as it has been in people. One study in humans demonstrated that over a 3 year period in patients (n=96) initially presenting for abdominal pain, 17 % of patients had complete resolution of sludge, 60% had a waxing and waning course, and 14% developed gallstones. Of those same patients, 12 % developed complications such as biliary colic and/or pancreatitis and underwent cholecystectomy. Compiled data from various studies on the progression of biliary sludge in people have shown that approximately 50% of patients with sludge presenting for abdominal pain will have resolution of biliary sludge, 20% will have persistent biliary sludge but remain asymptomatic, 10-15% will develop symptoms, and 10-15 % will develop gallstones.[59]

The chemical composition of spontaneous biliary sludge and concurrent GB histopathology in dogs has not been evaluated. Experimental studies have demonstrated consistent formation of biliary sludge and pigment stones in six weeks in dogs fed a lithogenic diet (low protein, high cholesterol, high carbohydrate, methionine deficient).[70,71] These changes can be reversed with a return to normal diet.[72] In these

studies, macroscopic and microscopic evaluation of sludge showed viscous material with green to black particulate material consistent with bilirubin particles in mucin. Dogs with diet-induced biliary sludge and pigment choleliths had various abnormalities within GB bile including increases in total and ionized calcium, unconjugated bilirubin, unconjugated bile salts, and mucin.[71-73] Histopathology revealed increased secretory vesicles and mucinous crypts analogous to cystic mucinous hyperplasia (CMH) of the GB epithelium.[70,72] In one dog with naturally occurring biliary sludge, the GB contained viscous bile with black to green particulate material which was similar to diet-induced biliary sludge.[27] Macroscopic evaluation of bile associated with mucoceles in eight dogs was consistent with previous reports of experimental sludge and pigment choleliths in dogs. The chemical composition of the bile in a single dog that had microscopic analysis of biliary sludge consisted of bilirubin particulates, calcium carbonate, and mucin.[46]

In summary, veterinary literature suggests that biliary sludge has a high prevalence in dogs and its clinical significance is unknown. Biliary sludge is typically found incidentally during abdominal ultrasonography. Indications for treatment and monitoring are yet to be determined. Further studies evaluating biliary sludge are warranted to determine its clinical significance in dogs.

c. Cystic Mucinous Hyperplasia

Cystic mucinous hyperplasia is characterized by a hyperplastic GB epithelium with cystic accumulations of mucus and papillary projections into the GB lumen.[1] Cystic mucinous hyperplasia may be apparent via abdominal ultrasonography and appears as sessile or polypoid masses along the GB wall.[1,4] The clinical significance of CMH is unknown, but is believed to contribute to GBM formation. Cystic mucinous hyperplasia

has been documented as an incidental finding at necropsy.[74] Cystic mucinous hyperplasia has been reported to be associated with administration of progestagens, cholecystitis, and GBM formation.[24,25,46,75,76,77] Cystic mucinous hyperplasia was histologically present in 100% of dogs with GBM in 4 studies,[24,25,46,78] and only present in 39% and 40% in two other studies.[79,80] It remains to be determined whether cystic mucinous hyperplasia is a primary or secondary occurrence with GBM.[1,76]

d. Gallbladder Mucocele

Gallbladder mucoceles are an accumulation of gelatinous to semi-solid mucus mass and inspissated bile distending the GB lumen.[24,27] Scientific reports of GBM in the dog are frequent over the past 10 years and, currently, GBM is the most common GB disease in dogs. Historically, GBM were considered an incidental finding at necropsy.[74] However, GBM are associated with biliary obstruction, cholecystitis, GB rupture, abdominal pain, vomiting, and mortality.[1,24,25,79,81,82] The etiology of GBM formation is unknown. Histologically, CMH and mucin with congealed bile filling the GB lumen are described with GBM. The association of CMH and GBM suggests either a primary or secondary dysfunction of mucus secreting glands leads to GBM formation. Mucus hypersecretion occurs secondary to exposure of the GB epithelium to concentrated bile acids, particularly hydrophobic bile acids. Inflammatory cytokines (tumor necrosis factor alpha and interleukin-1), prostaglandins, and lipopolysaccharides also lead to mucin hypersecretion and GB epithelial inflammation.[83-85] It is suspected that GB dysmotility also plays a role in GBM formation where biliary stasis, biliary sludge formation, and mucus retention occur. A mutation in the phosphatidylcholine floppase (ABCB4) gene is associated with cholestatic disease in humans.[86] This canalicular-membrane transporter

is responsible for providing phospholipids for the formation of micelles with bile salts. An insertion mutation of the ABCB4 gene in dogs was found to be associated with GBM in dogs, particularly in Shetland Sheepdogs.[87] However, a subsequent study with a larger group of dogs failed to find a strong association between ABCB4 insertion mutation and GBM.[88] The etiology of GBM is multifactorial; ABCB4 insertion mutation may be one potential risk factor.

Risk factors for GBM formation include gallbladder dysmotility, dyslipidemias, and endocrinopathies such as hyperadrenocorticism and hypothyroidism.[89-91] In one study, hypothyroidism was over represented in the GBM dogs versus control dogs suggesting a possible bias where dogs with GBM may have been more likely to be tested for hypothyroidism than control dogs.[89] Gallbladder mucoceles typically occur in older dogs (median age 10 years) with a breed predilection for Cocker spaniels, Shetland Sheepdogs, and Miniatures Schnauzers. [24,25,46,78,79,89,92] Clinical signs associated with GBM are generally nonspecific and may overlap with concurrent diseases. Clinical signs reported include vomiting, anorexia, lethargy, diarrhea, polyuria and polydipsia. Physical examination findings include abdominal pain, icterus, pyrexia, tachypnea, and tachycardia.[24,25,46,76,79] However, GBM may be found incidentally in dogs being evaluated for other diseases. Prevalence of dogs without clinical signs of hepatobiliary disease is reported between 23 – 44%.[24,25,82] The true prevalence of GBM in dogs with or without clinical signs is difficult to determine as signs can overlap concurrent disease and dogs with clinical signs related to GBM may be overrepresented as the majority of studies pertain to those undergoing surgical intervention. Clinicopathologic abnormalities in dogs with GBM include increased serum liver enzyme activity (ALT, ALP, GGT)

hyperbilirubinemia, hypercholesterolemia, hypertriglyceridemia, and leukocytosis and mature neutrophilia. [24,25,46,76,79] Common histologic findings of the GB in dogs with GBM include CMH (40-100%), ischemic necrosis (17-80%), infarction (21%), cholecystitis (10-50%) , and fibrosis (10-20%). Concurrent liver histopathology includes portal hepatitis (10-78%), cholangiohepatitis (30%), biliary hyperplasia (30-64%), cholestasis (20-37%), and vacuolar hepatopathy (2-43%).[24,25,46,78,79] Concurrent bacterial cholecystitis varies with infection rates ranging from 2% to 66%.[24,25,78,79] This wide range may be a reflection of non-standardized sampling and use of perioperative antibiotics.

Abdominal ultrasonography is the mainstay for imaging diagnosis of GBM. The classic ultrasound appearance of a GBM is described as a kiwi-like appearance of intraluminal GB contents with hyperechoic immobile striations of inspissated bile within hypoechoic mucus structures.[24] Various ultrasonographic appearances of GBM have been described. These include echogenic immobile biliary sludge filling the gallbladder, stellate pattern, and the classic kiwi-like pattern.[24,27,82] It is suggested that these may represent a continuum of early to mature mucoceles.[24] The incidence of GB wall thickening in dogs with GBM ranges from 5% to 30%.[24,46,79,82] Ultrasound is also used to determine the presence of GB rupture and/or EHBO of the cystic duct, hepatic ducts, or BD. A distended BD (>3mm) may support BD obstruction but this is not definitive. Dogs with BD dilations on ultrasound ranged from 9 to 60%, but it was not clear how many of these dogs had BD obstruction.[24,25,79,82] One study showed BD obstruction with congealed bile was present in 30% of cases at time of surgery.[25] In another study, all dogs had evidence of BD obstruction with mucus at the time of surgery or necropsy.[24]

There are no specific ultrasound findings to determine the presence of concurrent cholecystitis. Pericholecystic hyperechoic fat, pericholecystic fluid, discontinuous GB wall, and unidentifiable gallbladder with free-floating mucocele within the peritoneum all support the presence of GB rupture.[24,25,79] The specificity and sensitivity of ultrasound in determining GB rupture in dogs with GBM is 100% and 79-85% respectively. [25,82] Various ultrasonographic appearances of GBM were found not to be predictive of clinical signs, biochemical abnormalities, or presence of GB rupture.[82] Unless there are specific findings of GB rupture, ultrasound alone is not helpful in determining the clinical significance of GBM or treatment decisions.

Medical and surgical management have been proposed for treatment of GBM in dogs. When gallbladder rupture is present, emergency surgical intervention is warranted. When clinical signs and biochemical abnormalities are present, it is generally recommended that a cholecystectomy be performed in lieu of medical management.[24,25,82,93] There are various opinions within the veterinary literature about the management of clinically silent GBM. Cholecystectomy is generally recommended as CMH is present in the vast majority of dogs with GBM suggesting the GB itself may be diseased.[79] Gallbladder mucoceles can lead to pressure necrosis of the GB wall, thus, cholecystectomy can be performed prophylactically in stable patients to eliminate the risk of GB rupture and subsequent bile peritonitis. Gallbladder rupture was reported in 23%-61% of dogs with GBM at the time of surgery.[24,25,78,79,82] Evidence to support surgical versus medical management is weak as the majority of studies include dogs that underwent cholecystectomy only. Assessment of GB dysmotility via functional ultrasonography has been used by institutions to support the necessity of cholecystectomy

in cases of early GBM.[1] There is no evidence to support that surgical over medical management results in a better outcome in dogs with GBM with evidence of GB dysmotility. It is also unknown whether medical management of GBM improves GB motility. Perioperative mortality rates vary and range from 7% to as high as 40%.[25,78,79,94] Thus, the decision for surgical versus medical management should not be made lightly. Elevated serum lactate, septic peritonitis, elevated serum creatinine, and postoperative hypotension have been associated with increased risk of perioperative mortality in dogs undergoing biliary surgery including treatment of GBM.[24,25,78,82] Presence of GB rupture and bile peritonitis were not associated with overall survival, and long-term survival is possible in patients that survive the perioperative period. [79,81] Common complications postoperatively are pancreatitis and bile peritonitis.[46,79,80] Appropriate medical therapy for the management of GBM is unknown given the lack of studies assessing medical therapy as well as poor understanding of the underlying disease process. Currently accepted medical therapy includes UDCA, antimicrobial therapy, low fat diet, and treatment of concurrent diseases (hyperadrenocorticism, hypothyroidism, dyslipidemias), which are associated with GBM. Ursodeoxycholic acid reduces the toxicity of bile acids and GB mucin secretion, and has immunomodulatory, cytoprotective, and cholerectic properites.[95-97] There are a few cases of ultrasonographic resolution or improvement of gallbladder mucoceles with medical management with other cases having static disease.[46,82,98] Further investigations are needed to determine appropriate therapy (surgical versus medical) in clinically silent cases as well as ill dogs. Effective medical therapy in dogs with GBM also needs to be determined.

e. Cholecystitis

Cholecystitis is a broad term referring to inflammation of the GB. Cholecystitis is further characterized as acute or chronic, septic or nonseptic, calculus or acalculus, necrotizing and/or emphysematous.[1,2,6] Cholecystitis is an uncommon diagnosis in the dog. One study evaluated the prevalence of GB diseases in dogs, irrespective of health status, presenting to a referral hospital in India and found the prevalence of cholecystitis to be 2.3%.[63] This study utilized only ultrasonographic characteristics for the diagnosis of cholecystitis; therefore, the true prevalence may be under or overestimated in that population of dogs. The etiology of cholecystitis is poorly understood, but has been associated with infectious agents, systemic disease, trauma, choleliths, and neoplasia.[1,99-103] Biliary stasis, mechanical irritation from biliary sludge and choleliths, ascending bacterial infections, and mucoceles may be predisposing factors for cholecystitis.[1,23,99,104] Shetland sheepdogs may be predisposed to the development of cholecystitis.[46]

Cholecystitis has both acute and chronic presentations. As with most GB diseases, clinical signs are nonspecific and common to other gastrointestinal and systemic diseases.[2] Anorexia, vomiting, abdominal pain, and fever are typical signs of acute, severe cholecystitis, and patients may also present with signs of shock.[2,4,23] Conversely, patients with chronic cholecystitis have milder signs of chronic intermittent vomiting, anorexia, weight loss, abdominal pain, or no clinical signs at all. Necrotizing cholecystitis generally presents in the acute stage and is a common cause of GB rupture and bile leakage. Necrotizing cholecystitis is associated with septic inflammation and/or pressure necrosis, underlying immune suppression (drugs, hyperadrenocorticism, diabetes mellitus), and GB infarction. Infarction of the cystic artery without underlying

inflammation is associated with thrombus formation, atheromatous vascular change, and underlying endocrine disease.[3,99] Acute cases of cholecystitis can present with or without GB rupture, and chronic cases may present with cholecystitis adhesions and fistula formation.[2,3] Emphysematous cholecystitis is characterized by gas within the GB wall, and is usually associated with a septic process due to gas producing bacteria, *Clostridium perfringens* or *Escherichia coli.*[1,4,105-107] Diabetes mellitus has been associated with emphysematous cholecystitis in dogs as well as humans.[107]

Clinicopathologic abnormalities of cholecystitis are also nonspecific and variable. Common abnormalities include increased serum liver enzyme activity, hypercholesterolemia, and hyperbilirubinemia consistent with cholestasis and biliary disease, and leukocytosis with or without a left shift.[1,4] Abdominal radiographs may show a right quadrant abdominal mass effect or poor serosal detail if biliary leakage is present. Gas-filled GB or gas opacities in the pericholecystic region may be seen in cases of emphysematous cholecystitis.[1,15] Abdominal ultrasonography can be suggestive of cholecystitis as denoted by thickened, hyperechoic, irregular and/or laminar GB wall, echogenic intraluminal contents, pericholecystic fluid or echogenic abdominal effusion. However, these findings are not specific for cholecystitis and may be seen with other gallbladder diseases or systemic disease such as edema, hypoproteinemia, and pericholecystic effusion.[15,23,108] A "Murphy's sign" (pain induced from pressure of the ultrasound transducer when it is applied to the ventral abdomen) may be elicited in affected dogs.[109] Cholecystocentesis aids in the diagnosis of cholecystitis. Aspirated bile can be evaluated cytologically for inflammation and infections agents and submitted for bacterial culture and sensitivity. Common bacterial isolates include *Escherichia coli,*

Entercoccocus sp., Streptococcus sp., Klebsiella sp., Clostridium sp., and *Bacterioides sp*.[2,4,6,23,31] Bile in healthy dogs is usually sterile; however, one study did identify subclinical bactibilia in a small percentage of healthy dogs. [110]

Treatment includes medical management and may necessitate surgical intervention depending on severity of signs and presence of emphysematous cholecystitis and/or GB rupture. Medical therapy includes antimicrobial administration, ideally based on culture and sensitivity, UDCA, and supportive care.[1,23] Cholecystectomy is usually the surgical treatment of choice. If only the GB is affected, then cholecystectomy may be curative.[1,3] In some cases biliary diversion procedures may be necessary depending on involvement of the common bile duct.[1]

f. Cholelithiasis

Choleliths are stones within the biliary system. Cholecystoliths refer to stones within the GB whereas the term choledocholith refers to stones within BD, hepatic ducts and interlobar ducts.[2,4,99] Cholecystoliths are more common than choledocholiths. Choleliths have been reported to contain calcium salts, bilirubin, and cholesterol.[1,4,103,111] Pigment stones are the most common type in dogs with mixed stones and cholesterol stones being less frequent.[1,103,111] Cholesterol stones are common in humans. The difference in frequency and composition of choleliths in humans versus dogs may be related to differences in bile composition.[1] Protective factors for dogs include absorption of calcium from the gallbladder, lower cholesterol content in bile, high saturation index of cholesterol, and antinucleating factors reducing supersaturation of calcium salts.[1,112,113] Pigment stones are called black or brown depending on the degree of bilirubin particles and calcium bilirubinate respectively. Cholecystitis and biliary stasis are predisposing

factors for brown-pigment stones. Inflammation, mucin hypersecretion, cellular debris, bilirubin deconjugation, and biliary stasis serve as a nidus for crystallization.[1,3] Deconjugation of bilirubin can occur non-enzymatically or via beta-glucuronidase. Unconjugated bilirubin becomes available to form calcium bilirubinate particles. Aggregation of calcium bilirubinate is potentiated by bacterial colonization, mucin secretion, and biliary stasis.[1,60] Supersaturation of calcium salts and increases in ionized and total calcium in canine GB bile also play a role in the formation of pigment choleliths.[73,114] Experimental BD ligation promoted the formation of biliary sludge and pigmented choleliths in dogs.[64] Gallbladder distension leads to mucin hypersecretion, creating available mucin to complex with bilirubin forming biliary sludge and stone formation.[1,60] Likewise, cholelithiasis can lead to biliary stasis and bacterial infection by mechanical irritation to GB and ductal epithelium. The role of diet in the formation of cholelithiasis in dogs has not been explored, but experimentally-induced pigment stones have developed in dogs fed a low protein, high cholesterol, high carbohydrate, methionine deficient diet.[70] Middle-aged to older, female, small breed dogs are predisposed to choleliths, and Miniature Poodles and Miniature Schnauzers have been reported to have an increased incidence.[1,3,103,115,116]

Choleliths are typically clinically silent in dogs with most found incidentally during abdominal ultrasonography or necropsy. Choleliths are clinically significant if associated with extrahepatic biliary obstruction and cholecystitis. Clinical signs include anorexia, vomiting, fever, icterus and abdominal pain.[2,103] Signs may be acute and severe or chronic and intermittent. Affected dogs can have increased serum liver enzyme activity, hyperbilirubinemia, and hypercholesterolemia depending on the clinical

manifestation of cholelithiasis. Hyperbilirubinemia is present in cases of EHBO or related sepsis.[1,2] If concurrent cholecystitis is present, leukocytosis and left shift may be identified. Abdominal radiography may disclose mineral opacities within the biliary structures suggestive of cholelithiasis. Most choleliths do not contain enough calcium content to be radiopaque limiting the use of radiography for the detection of cholelithisasis.[2,15] Ultrasonography can detect stones that are 2 mm or greater in size.[1] Ultrasonography is also helpful in detecting concurrent extrahepatic biliary obstruction and possibly cholecystitis.

Treatment of choleliths involves medical and/or surgical management. Medical dissolution of choleliths is unsuccessful in dogs and cats unlike in humans where cholesterol stones are more common and amenable to dissolution with UDCA. Medical therapy in dogs and cats includes the use of UDCA, s-adenosylmethionine (SAMe), antimicrobials, vitamin E, and anti-inflammatory medications based on results of liver histopathology. Medical management is more likely to be successful in small ductal choleliths.[1] Surgery is the treatment of choice with concurrent cholecystitis and common bile duct obstruction. Cholecystectomy is prefer over cholecystotomy given the lower morbidity and mortality reported with the former procedure.[3,103] The BD should be assess for patency and flushed. If the BD cannot be unobstructed, choledochotomy, temporary stent placement or biliary-enteric anastomosis may be warranted.[1,3]

D. Gallbladder Disease in Humans

Gallbladder disease in humans encompasses a wide range of disorders that vary in severity of clinical manifestations. Cholelithiasis, biliary sludge, cholecystitis, biliary dyskinesia, and neoplasia are diseases associated with the GB in people.[43,44,59,117] Biliary

sludge can lead to the formation choleliths; however, both are commonly asymptomatic. Symptomatic biliary sludge and cholelithiasis are related to concurrent cholecystitis, pancreatitis, cholangitis, and ductal occlusion.

a. Biliary Sludge

Biliary sludge is an accumulation of bilirubinate particles, calcium salts, cholesterol monohydrate crystals, and mucin.[118] Microlithiasis is another term used to describe biliary sludge, particularly stones less than 3mm. The prevalence of biliary sludge ranges from 0 to 0.3% in healthy adults, 1.7 % in asymptomatic patients undergoing routine abdominal ultrasound for medical evaluation, and 6.7% in patients undergoing abdominal ultrasound for abdominal pain.[118-120] Biliary sludge is considered clinically significant as it can predispose to the development of cholelithiasis, acute idiopathic pancreatitis, acalculus cholecystitis, and biliary colic.[90,118,120-123]

Natural history studies have shown biliary sludge can resolve, wax and wane, persist, or progress to clinical disease.[58,59,120,124] In one retrospective study evaluating patients with biliary sludge without concurrent cholelithiasis or other GB abnormalities, 19% developed choleliths and/or acute cholecystitis.[120] This is in comparison to 10% of the general adult US population and 25% of those over 50 years of age who will go on to develop gallstones.[44,117] Various studies on the natural history of biliary sludge suggest that 50% of symptomatic patients will have spontaneous resolution of biliary sludge, 20% will develop asymptomatic biliary sludge, and 25-30% will develop complications such as cholelithiasis or biliary pain.[59] Risk factors for the formation of biliary sludge include pregnancy, diet, rapid weight loss, drug administration (octreotide, ceftriaxone), prolonged fasting, parenteral nutrition, and organ transplantion.[59,60,119,125-132]

The chemical composition of biliary sludge depends on the underlying cause. Biliary sludge composition can include cholesterol monohydrate crystals, calcium bilirubinate, calcium salts, and mucin glycoproteins. Unconjugated bilirubin and mucin in GB bile has been shown to increase in people with biliary sludge and pigment choleliths. This less soluble form of bilirubin binds to mucin and calcium ions leading to pigment particulate formation within GB bile.[58-60,133] The morphologic changes in GB epithelium in people with biliary sludge include mucus hypersecretion, glandular metaplasia, and cholesterolosis (abnormal deposition of lipid within the lamina propria of the gallbladder wall).[60,118] The GB pathology associated with biliary sludge suggests that it is not a benign process.

Diagnosis of biliary sludge is determined via abdominal ultrasound, endoscopic ultrasound, endoscopic retrograde cholangiography, and bile microscopy. Echogenic, gravity dependent, non-shadowing GB contents are the characteristic findings in biliary sludge.[46,59,118] Abdominal ultrasonography has a 55-65% sensitivity and 96% specificity in the detection of biliary sludge. Endoscopic ultrasonography is utilized in cases where biliary sludge is suspected but not apparent on routine abdominal ultrasonography. Endoscopic ultrasonography has a sensitivity and specificity of 92-96% and 86-100% respectively.[59,119] Bile samples can be obtained via endoscopic retrograde cholangiography and evaluated via polarized light microscopy. Microscopy allows for composition analysis of microlithiasis and is considered the gold standard for diagnosis.[59,119]

Treatment of biliary sludge depends on the clinical presentation. Asymptomatic patients can be monitored for clinical signs without institution of therapy. In

symptomatic patients, therapy is aimed at resolution of the underlying cause along with surgical or medical therapy. In cases of biliary pain, cholecystitis, and pancreatitis, cholecystectomy is recommended.[59,60,119] Endoscopic sphincterotomy of the SOD can be performed in patients with recurrent cholangitis and pancreatitis.[60] Medical management includes dissolution therapy with UDCA, CCK injections in patients receiving total parenteral nutrition, and in some cases percutaneous cholecystocentesis for biliary draining and flushing.[59,60,95]

b. Cholelithiasis

Cholelithiasis affects about 10-15% of the adult population in the United States.[44,117] The prevalence of gallstones varies depending on ethnicity and geographic location. About 80% of people with gallstones are asymptomatic.[44,45] These so-called "silent" stones are found incidentally. Symptoms may arise at a later time, but the risk is considered low. However, cholecystitis, cholangitis, pancreatitis, or common bile duct obstruction can cause severe signs of abdominal pain, nausea, and vomiting.[44,117]

There are three categories of choleliths based on composition: primary cholesterol stones, pigment stones, and mixed stones. [44,45] In the United States, 70-90% of stones are cholesterol based with the rest being black pigment stones. Brown pigment stones occur mainly within the common bile duct.[134] Increased cholesterol and calcium content in bile, shifts in bile salts, decreased phospholipids, and increased mucin results in biliary sludge and cholelith formation.[45,59,60,117] Risk factors for stone formation include age, female sex, obesity, genetics, diet, pregnancy, rapid weight loss, medications, biliary sludge, biliary stasis, and dyslipidemias.[44,45,124,135] Choleliths are diagnosed via transabdominal ultrasound, endoscopic ultrasound, endoscopic

cholangiopancreatography, cholescintigraphy, and/or magnetic cholangioportography. Ultrasonography is most commonly utilized in the diagnosis.[45] Cholecystectomy is the treatment of choice in symptomatic patients. Medical management with UDCA and diet are reserved for patients who are not good candidates for surgery.

CHAPTER II: Natural History of Biliary Sludge in Dogs

A. Introduction

Sludge within the gallbladder (GB) is commonly seen with ultrasonographic examination and is defined as the presence of gravity-dependent, non-shadowing, echogenic material within the lumen of the GB[24,57,118] The prevalence is not different among healthy dogs, dogs with hepatobiliary disease, or dogs with other diseases.[57] However, in the only study that evaluated GB motility in dogs with biliary sludge, those with sludge were shown to have decreased GB motility when compared to dogs without biliary sludge, suggesting that biliary sludge may not be a benign process.[47] Biliary stasis and modifications to bile within the GB promote the formation of biliary sludge.[59,60] An in vitro study using cultured canine GB mucosa cells revealed that increased hydrophobic bile acids within bile stimulate mucus secretion from the GB epithelium.[136] This suggests that prolonged exposure to concentrated bile and/or biliary sludge could cause mucinous hyperplasia (CMH). The pathogenesis of gallbladder mucocele (GBM) formation is unknown, but GBM are associated with CMH and GB dysmotility.[24,25,46,47] Few causes and risk factors for biliary sludge formation in dogs have been identified. Dyslipidemias and endocrinopathies are associated with GBM and are speculated to promote biliary sludge formation.[46,47,89,98] Cocker spaniels were shown to have a higher prevalence of biliary sludge[62] as well as an increased incidence of GBM[24,25,79] thus supporting a theory of a continuum of disease from sludge to mucocele. Biliary stasis and GB dysmotility, biliary sludge formation, and CMH may represent a disease continuum with formation of a GBM as the end stage of the disease process.

Dogs with GBM can develop cholecystitis (bacterial or sterile), and GB necrosis and rupture, which are life-threatening and require emergent surgical intervention.[24,25,79] Thus it is important to identify dogs with GB disease earlier in the course of disease to avoid potentially serious complications. Studies evaluating the natural progression of biliary sludge in dogs are lacking, as biliary sludge has not been evaluated over any period of time in dogs. In addition, the authors are not aware of any studies that have identified risk factors for developing serious GB diseases such as cholecystitis and GBM in dogs with biliary sludge. If biliary sludge is linked to GBM formation or cholecystitis, biliary sludge should be considered clinically significant and treatments should be developed to reduce the progression of sludge formation.

The specific aims of this study were twofold: First, to determine the natural history of biliary sludge in apparently healthy dogs using serial ultrasonographic and serum biochemical examinations during one-year. We hypothesized that biliary sludge would increase over time and, in some dogs, become solid and immobile, which are features of GBM. We also hypothesized that dogs with progressive sludge would have alterations in biochemical parameters and increasing GB volumes. Second, to determine the histopathological changes of the gallbladder and liver associated with biliary sludge. We hypothesized that dogs with biliary sludge would have a higher prevalence of CMH and/or cholecystitis than dogs without biliary sludge.

B. Materials and Methods

Seventy-seven healthy, student and staff-owned dogs ≥ 4 years of age were screened for biliary sludge via transabdominal ultrasonography at the Virginia-Maryland College of Veterinary Medicine (VMCVM). Institutional Animal Care and Use

Committee approval and informed owner consent were obtained. Healthy was defined as the absence of illness within the preceding three months, lack of chronic drug administration, and an unremarkable physical examination. Biliary sludge was defined as gravity dependent, echogenic material within the gallbladder. The 45 dogs in which biliary sludge was identified were evaluated every three months for a 12-month period. Three dogs with biliary sludge were ultimately excluded from final analyses due to chronic medication administration and/or the development of serious disease not associated with the biliary tract. Each evaluation included a physical examination, serum biochemistry, and hepatobiliary (liver, pancreas, gallbladder) ultrasound. Dogs were fasted for at least 12 hours prior to ultrasound examination.

Ultrasound examinations were performed and/or supervised by a board-certified radiologist using an ultrasound machine (Philips iU22, Philips Medical Systems, Bothell, WA) equipped with a preset broad bandwidth operating frequency transducer (8–5 mHz microconvex). Transverse and longitudinal images of the gallbladder were obtained with dogs in dorsal and/or lateral recumbency via subcostal and/or right-sided intercostal approaches. Gallbladder dimensions included height (H) and length (L) obtained from longitudinal images, and width (W) and depth (D) obtained from the transverse images. Gallbladder dimensions were determined from three transverse and three longitudinal images and the average measurements were recorded. Gallbladder volumes (GBV) were calculated using the ellipsoid method (Volume = 0.53 x L x W x D).[19] Relative GBV was calculated by dividing the GBV by kilograms of body weight (GBV/kg).[21] Gallbladder wall (GBW) thickness was measured in a standard location of the body of the GB[57] and any abnormalities in the structure of the gallbladder were documented. In dogs

where the GBW was poorly visualized, a measurement of 1 mm was recorded. Relative sludge, percentage of biliary sludge filling the GB, was assessed using images with maximum GB area on the longitudinal image. Biliary sludge area and GB area were determined using imaging software (OsiriX v.6.0.2, Pixmeo Sarl). Relative sludge, was calculated as follows: (sludge area/GB area) x 100. Degree of biliary sludge was scaled using a score of 0-4: 0 = absence of sludge (0%), 1 = mild (0.01%-24.4%), 2 = moderate (24.5%-49.4%), 3 = moderate to severe (49.5%-74.4%), 4 = severe (74.5%-100%) (Figure 1). Gallbladder contents were evaluated based on gravity dependency. Gallbladder contents were categorized as: 0 = dependent sludge, 1 = dependent and non-dependent sludge, 2 = non-dependent (sludge adhered to non-dependent GB wall only), 3 = dependent and suspended sludge, 4 = suspended sludge (sludge that is not adjacent to the GB wall), 5 = suspended, dependent, non-dependent sludge (Figure 2). At the end of the study dogs were classified as having persistent (sludge present at each examination whether decreased, static, or increased), resolved (absence of sludge at all subsequent examinations), or recurrent sludge (disappearance and reappearance of sludge at various examinations). Degree of biliary sludge using a score of 0-4 at initial and final examinations were used to categorize dogs as having decreased, static, or increased biliary sludge.

Blood was collected from each dog after each examination, and the following parameters measured in serum: activities of alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), and concentrations of total bilirubin, cholesterol, triglycerides, albumin, and total calcium.

In the second portion of this study, histologic analysis of the liver and GB was performed on samples collected from dogs that were euthanized or died from spontaneously occurring diseases within the VTH. Dogs were enrolled in this study if they died or were euthanized, undergoing subsequent necropsy, and owners consented to post-mortem sample collection. Ultrasound examination for the presence or absence of biliary sludge was performed within 30 minutes of death. An approximately 2 x 3 cm wedge liver biopsy was collected from either the right medial or quadrate liver lobes along with complete GB removal performed within 30 to 60 minutes of death. Samples were fixed in 10% neutral buffered formalin, routinely processed with hematoxylin and eosin stain, and examined by a board-certified veterinary pathologist. Sections of GB and liver were evaluated for cystic mucinous hyperplasia, cholecystitis, intrahepatic cholestasis, cholangitis, steroid-induced hepatopathy, and hepatitis according to guidelines established by the World Small Animal Veterinary Association.[137,138]

Statistical analysis

Mixed model ANOVA and Friedman chi-square tests were performed to detect changes in degree of sludge, relative sludge, GBV, GBV/kg, and biochemical indices over time. Mantel-Haenzsel chi-square tests were used to assess significant difference in changes in GB contents over time. Kruskal-Wallis test was performed to determine differences in biochemical indices, GBW thickness and GBV among dogs grouped according to changes in degree of sludge over time. Normal probability plots showed that age, relative sludge, calcium, cholesterol, GBV, GBV/kg were normally distributed while degree of sludge, ALT, ALP, total bilirubin, GGT, triglycerides, albumin, and GB wall thickness were skewed. Gallbladder content was inherently categorical. Normal

probability plots showed that ALT, ALP, total bilirubin, GGT, triglycerides, albumin, total calcium, GBV and GB wall thickness were skewed in dogs with increased, decreased, static, and recurrent sludge. The mean values and standard deviation are listed for normally distributed data, and the median values and range are listed for skewed data. Significance was determined at $P < 0.05$.

C. Results

Forty-two dogs with biliary sludge were evaluated every 3 months for one year. All 42 dogs completed evaluations at 0, 3, and 6 months. Forty-one dogs were available at 9 months, and 38 dogs at 12 months. A total of 4 dogs did not complete the study because the owners and dogs were unavailable for scheduled recheck examinations. The mean age of dogs with biliary sludge was 6.4 ± 2.4 years. Twenty dogs were neutered males, 21 dogs were spayed female, and one dog was an intact male. Twenty-one dogs were mixed-breed, 3 were Pit bull Terriers, 3 were Dachshunds, 2 were Boston terriers, 2 were Boxers, 2 were Great Dane, and 2 were Chihuahuas. None of the dogs developed clinical signs attributable to hepatobiliary disease during the study period.

Degree of Biliary Sludge

Initially, the degree of biliary sludge was mild, moderate, moderate to severe, and severe in 50% (21/42), 36% (15/42), 9% (4/42), and 5% (2/42) of dogs, respectively (Table 1). Upon conclusion of the study, biliary sludge was absent, mild, moderate, moderate to severe, and severe in 3% (1/38), 34% (13/38), 47% (18/38), 13% (5/38), and 3% (1/38) of dogs, respectively (Figure 3). The median degree of biliary sludge did not change significantly over time ($P = 0.36$). When analyzed as a continuous variable, mean relative sludge was not significantly different overtime ($P=0.45$) (Table 4).

Based on overall degree of biliary sludge (initial examination compared to final examination), it was persistent, resolved, and recurrent in 88% (37/42), 2% (1/42), and 10% (4/42) of dogs, respectively. Biliary sludge decreased, increased, or remained unchanged in 19% (8/42), 29%(12/42), and 40% (17/42) of dogs, respectively.

Gallbladder Content

The prevalence of dependent biliary sludge in the 74 dogs evaluated in this study was 57%. At initial examination, GB contents were 0, 1, 3, and, 5 in 64% (27/42), 2% (1/42), 24% (10/42), and 10% (4/42) of dogs respectively. Upon conclusion of the study, GB contents were 0, 1, 3, 4, and 5 in 32% (12/37), 3% (1/3 7), 31% (11/37), 3% (1/37), and 32% (12/37) of dogs, respectively. Frequency of GB contents did not change significantly over time (P=0.25) (Table 2). Only two dogs with an initial GB content of 1 or 5 had a GB content of 0 or 3 at the end of the study. Three dogs that had a GB content of 1 or 5 continued to have similar GB contents at the end of the study. Ten of 42 dogs that had GB content of 0 or 3 had a GB content of 5 by the end of the study (Table 6). Of the 10 dogs that developed GB content score of 5 at the end of the study, one dog had decreased degree of biliary sludge, 5 had a static degree of biliary sludge, 2 had an increased degree of biliary sludge, and 2 dogs had recurrent biliary sludge. Of the 12 dogs that had an increased degree of biliary sludge at the end of the study, 3 dogs developed GB content of 5 and the remaining 9 dogs continued to have a GB content of 0 or 3.

Gallbladder Measurements

Initial and 12-month mean GBV/kg were 1.02 ± 0.59 mL/kg and 1.20 ± 0.73 mL/kg respectively (Table 4). The median GBV/kg was not significantly different over

time (P=0.19). The mean GBV was also not significantly different (P = 0.54). Gallbladder wall thickness was poorly visualized in 47% (20/42), 64%(27/42), 62% (26/42), 63% (26/41), 63% (28/38) of dogs at 0, 3, 6, 9, and 12 months, respectively. Thus, those dogs were given a standard measurement of 1 mm. Initial and 12-month median GBW thickness was 1 mm (range 0.44 – 1.53 mm) and 1 mm (range 0.9-1.35 mm) respectively, and was not significantly different (P =0.81) (Table 4). However, GBW thickness was significantly different (P= 0.015) when comparing initial examination to 6 months and 9 months examinations (Table 4). Gallbladder volume and GBW thickness were not significantly different among dogs with recurrent, persistent, decrease, or increased degree of biliary sludge.

Biochemical Markers of Hepatobiliary Disease

There was no significant difference in serum activities of ALT, ALP, GGT, and concentration of total calcium, cholesterol, and triglycerides over time. There was a significant difference in median concentration of total bilirubin (P=0.002*)* and albumin (P=0.005), however, the median values were still within reference range (Table 3). Only one dog had consistently low serum albumin concentrations. When comparing biochemical markers of hepatobiliary disease among dogs categorized as having recurrent, persistent, decreased or increased sludge, there was only a significant difference in median total calcium concentration in which dogs with increased biliary sludge had lower median total calcium concentration compared to dogs with decreased or static sludge (P=0.024). The medians total calcium concentrations for all groups were within reference range (Table 5).

Gallbladder and liver histology

A total of 31 were screened for the presence or absence of biliary sludge via transabdominal ultrasound. The mean age was 9.2 ±4.2 years. Final diagnoses included neoplasia (45%, 14/31), hepatobiliary disease (9%, 3/31), neurologic disease (9%, 3/31), respiratory disease (9%, 3/31), trauma (2%, 6/31), toxicity (6%, 2/31), urologic disease (3%, 1/31), hemolymphatic disease (3%, 1/31), and gastrointestinal disease (3%, 1/31). At the time of death or euthanasia, 87% (27/31) dogs had biliary sludge. Of those with biliary sludge, 37% (10/27) and 67% (18/27) of dogs had cholecystitis and CMH respectively (Table 7). The severity of CMH in dogs with biliary sludge was mild, moderate, and severe in 50% (9/18), 40% (7/18), and 11% (2/18), respectively. The severity of cholecystitis in dogs with cholecystitis and biliary sludge was mild, moderate, and severe in 70% (7/10), 20% (2/10), 10% (1/10), respectively. Lymphoplasmacytic inflammation was present in all dogs with cholecystitis with one dog having concurrent neutrophilic inflammation. Of the four dogs without biliary sludge, two had mild lymphoplasmacytic cholecystitis and one had severe cystic mucinous hyperplasia. Liver histology was abnormal in 80% (25/31) of dogs. Most common histopathological diagnoses included steroid hepatopathy (15/31), biliary hyperplasia (6/31), neoplasia (6/31), and lymphoplasmacytic (1/31) or reactive hepatitis (2/31) (Table 8).

D. Discussion

Biliary sludge is currently regarded as an incidental finding with a high prevalence in healthy dogs.[57] The findings of this study corroborate that a high prevalence of biliary sludge (57%) occurs in healthy dogs ≥ 4 years of age or older. A previous study found a mean age of 4 years in dogs with biliary sludge.[69] As the goal of this study was to evaluate biliary sludge over time in dogs, a cutoff of 4 years of age was

used in this study to increase the likelihood of identifying dogs with biliary sludge. To the authors' knowledge, the spontaneous course of biliary sludge in dogs has not been systemically evaluated. The present study found that biliary sludge persists in 88% of dogs over the course of one year.

The median degree of biliary sludge at each examination was not significantly different over one year, but the degree of biliary sludge increased in 29% of all dogs. Dogs with an increased degree of biliary sludge were not the same as the dogs that developed non-dependent sludge. The findings of this study are in contrast to those in people with biliary sludge. A prospective study following human patients (n=96) for a period of 3 years found biliary sludge resolved in 17.7%, and disappeared and reappeared in 60.4%. Of those same patients, 8.3% had persistent sludge with asymptomatic gallstone formation, and 12.6 % developed clinical signs related to gallstone formation and biliary colic.[118]

Non-dependent or organized sludge would be expected in dogs prior to development of a GBM and dogs with GB content of 1, 2, or 5 may represent dogs that are developing immobile and/or organized sludge. Despite the majority of dogs having persistent biliary sludge, there was no significant change in GB contents overtime. A subset of dogs, 24%, with initial dependent biliary sludge developed a combination non-dependent and dependent sludge by the end of one-year period. This may have been due to changes in viscosity of biliary sludge, possibly due to increased mucin concentration in the bile, affecting how fast biliary sludge moves from the non-dependent to the dependent gallbladder wall.[139] Studies evaluating the composition of biliary sludge in dogs may help elucidate whether increased mucin is a feature of non-dependent biliary sludge.

The mean GBV/kg in dogs at the end of this study was 1.2 mL/kg (±0.73 mL/kg) but a significant change in GBV/kg was not found. Dogs typically have fasting GBV ≤ 1 mL/kg.[21] Further assessment of GB motility in dogs with GBV > 1 mL/kg would require calculation of an ejection fraction. This can be performed using ultrasonography to evaluate GBV at 1 and 2 hours after a test meal or erythromycin administration. Normal ejection fraction should be > 25%. [21] Dogs with biliary sludge and GBM had larger GBV and decreased GB emptying compared to controls in another study.[47] Whether the dogs in the present study with a GBV > 1 mL/kg had decreased GB emptying cannot be determined as ejection fractions were not calculated. Further studies assessing GB motility in dogs with progressive biliary sludge are warranted to see if it is associated with GB dysmotility in otherwise asymptomatic dogs. If progressive biliary sludge is found to be associated with GB dysmotility, evaluation of GB motility could potentially be utilized to assess the risk for progressive sludge and/or GBM formation.

There was a significant increase in GBW thickness at 6 and 9 months, but most of the dogs had normal GBW thickness (<2mm). In dogs, the normal GBW is either poorly visualized or appears as thin hyperechoic line, and the ability to accurately measure GBW depends on the angulation of the sound beam and degree of GB distension.[26] Approximately half the dogs in this study had a poorly visualized GBW at each examination period. Therefore, the significant difference in GBW thickness in dogs at 6 months and 9 months may not be a reflection of variation in GBW thickness, but rather variability in visualization of the GBW with ultrasound. Regardless, GBW thickness was not significantly different at the 12-month examination, making changes at 6 and 9 months unimportant.

Biochemical indices suggestive of cholestasis and hepatobiliary disease did not occur. Other studies have also found a lack of association between biliary sludge and changes in biochemical indices.[57,62] However, one study found higher serum cholesterol concentrations in dogs with biliary sludge compared to controls, but some dogs had concurrent diseases or were administered medications that could have affected their serum cholesterol concentrations.[47]

Dogs with biliary sludge at time of death or euthanasia in this study had a high prevalence of cystic mucinous hyperplasia, lymphoplasmacytic cholecystitis, and steroid-induce hepatopathy. Whether these findings are associated with the presence of biliary sludge or a consequence of age, concurrent diseases, or medication cannot be determined from this study. Only 4 dogs without biliary sludge were identified limiting our ability to evaluate the association of biliary sludge and the above histopathology findings. A larger number of dogs is likely necessary in order to obtain a similar number of dogs with and without biliary sludge for comparison.

There are several limitations to this study. The duration of this study was one year. It is possible that significant change in biliary sludge may take longer than one year to manifest. However, this is the first study to evaluate biliary sludge in dogs over any duration of time and supports the need for further studies evaluating biliary sludge over a longer period of time to assess for significant change in sludge volume and GB contents. When dogs were separated into groups based on the progression of biliary sludge, the numbers of dogs in each group were small. A larger study population may be necessary to detect significant differences in dogs with persistent or progressive biliary sludge versus dogs where biliary sludge resolved. Dogs were not rotated in position during

ultrasound examinations to assess for mobility of sludge. This could have led to misidentification of non-dependent biliary sludge in some dogs. However, in most cases biliary sludge was visualized gravitating to the dependent portion of the GB at the beginning of the ultrasound examinations. In order to allow for better visualization of the GB and presence of sludge, the position and placement of the ultrasound probe varied depending on body conformation. This may have led to variability in GB measurement and assessment of relative sludge area. Additionally, changes in GBV could affect relative sludge independent of biliary sludge area as both GB area and sludge area were used in the calculation. However, changes in GBV did not appear to influence the outcome of relative sludge in this study.

CHAPTER III: Conclusions

Biliary sludge did not significantly increase or become immobile over time in healthy dogs over the period of one year. It is possible that dogs in which biliary sludge persists could go on to form GBM if other risk factors associated GBM are present, such as breed, GB dysmotility, dyslipidemias, or endocrine disease. It is also possible that the progression of biliary sludge to GBM is insidious, taking longer than one year to cause significant changes in the relative amount of biliary sludge or gravity dependency. When considering the presence of biliary sludge in healthy dogs over one year, it does not appear to be associated with significant ultrasonographic GB abnormalities. Increased percentage of biliary sludge occurred in 29% of dogs and 24% of dogs went on to develop non-gravity dependent GB contents. These two groups may represent dogs that develop GBM perhaps due to changes in bile composition or GB dysmotility. These two subsets of dogs require further investigation over a longer period of time to assess the progression of biliary sludge volume and character.

In conclusion, biliary sludge has a high prevalence and persists in healthy dogs. This was the first study to evaluate the changes in spontaneously occurring biliary sludge as well as gravity dependency of biliary sludge over time. Additional studies assessing GB motility as well as the composition of spontaneously occurring biliary sludge in dogs are warranted and may provide information on pathophysiology of biliary sludge in dogs.

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FIGURES

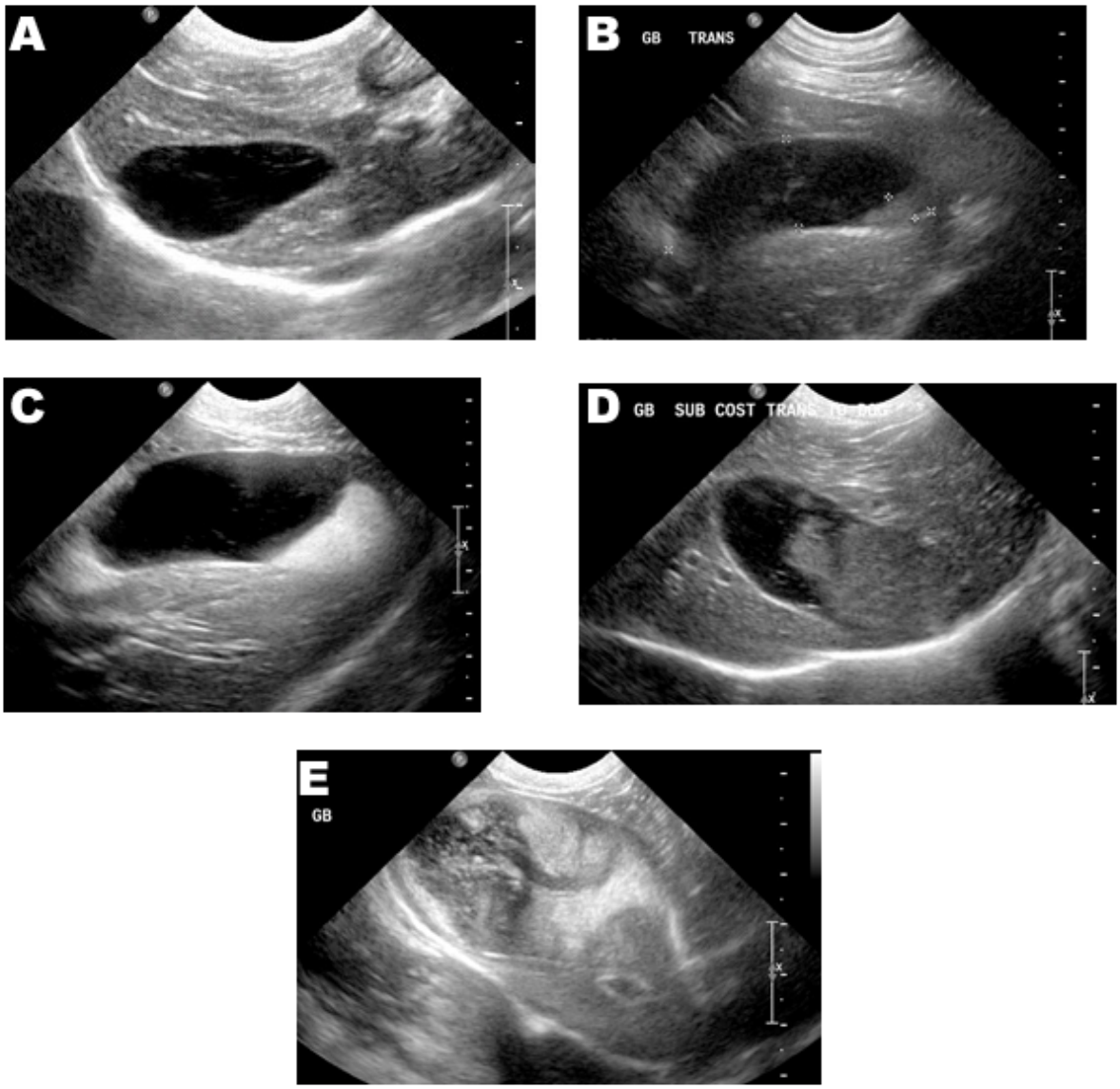

Figure 1. Degree of biliary sludge. A. absent biliary sludge score 0, B. mild biliary sludge score 1, C. moderate biliary sludge score 2, D. moderate to severe biliary sludge score 3. E. severe biliary sludge score 5

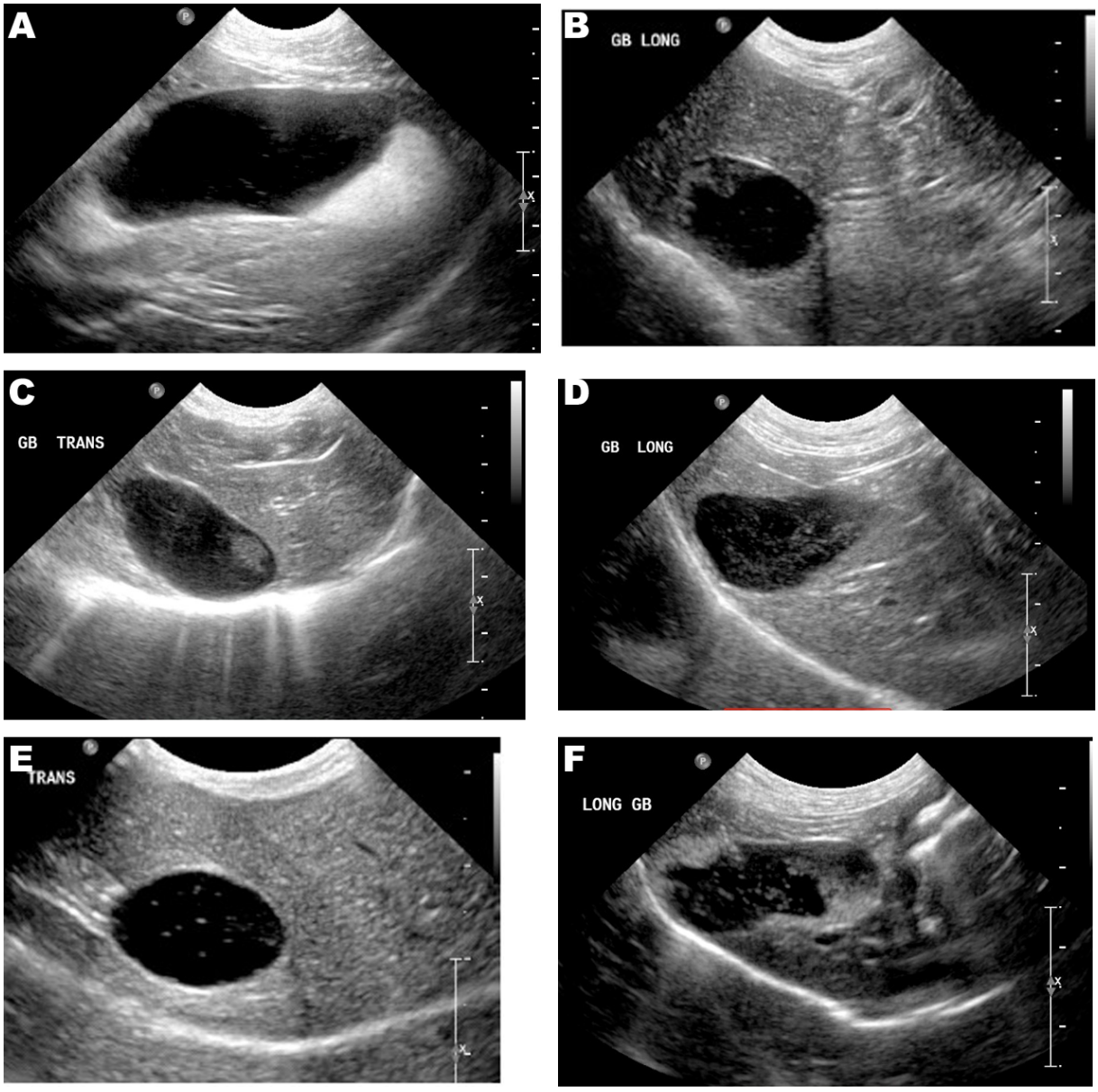

Figure 2. Gallbladder contents. A. GB content 0, B. GB content 1, C. GB content 2, D. GB content 3, E. GB Content 4, F. GB content 5.

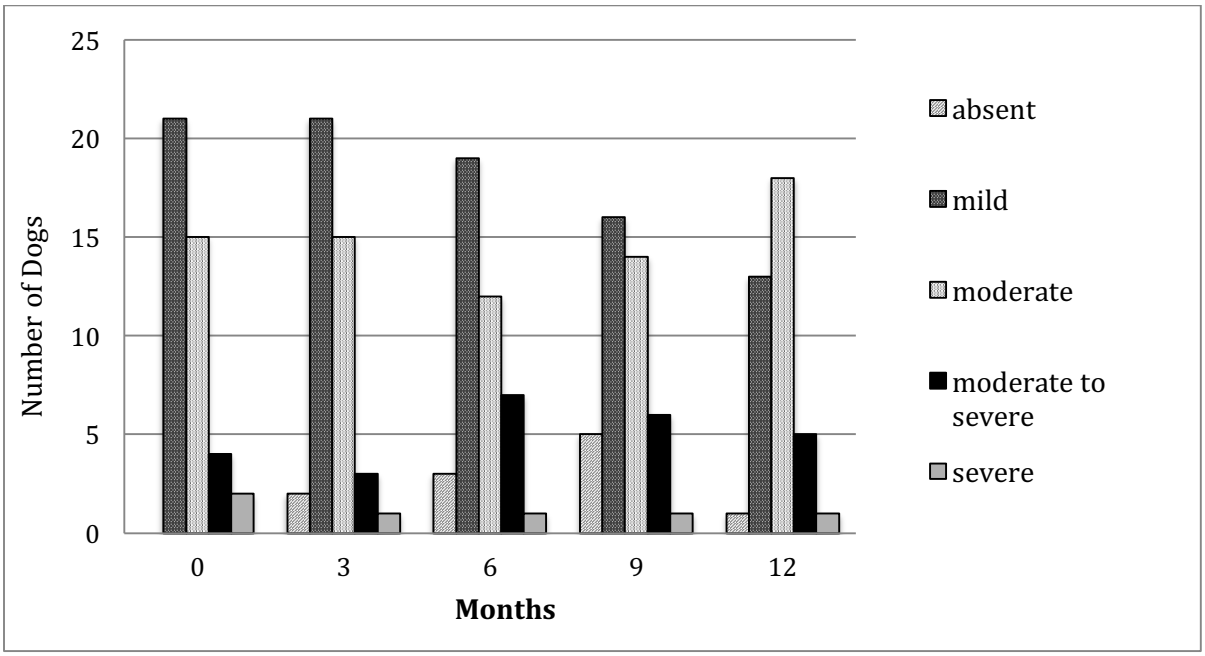

Figure 3. Degree of biliary sludge over 12-month period

TABLES

Table 1. Degree of biliary sludge in dogs over 12-month period

Degree of sludge	Initial n (%)	3 mo. n (%)	6 mo. n (%)	9 mo. n (%)	12 mo. n (%)
	42	42	42	41	38
0	n/a	2 (5)	3 (7)	5 (10)	1 (3)
1	21 (50)	21 (50)	19 (45)	16 (39)	13 (34)
2	15 (36)	15 (36)	12 (29)	14 (34)	18 (47)
3	4 (9)	3 (7)	7 (17)	6 (15)	5(13)
4	2 (5)	1 (2)	1 (2)	1 (2)	1 (3)

n/a – not applicable as dogs were excluded for lack of sludge at initial examination, n= number

Table 2. GB contents in dogs with biliary sludge over 12-month period

GB contents	Initial n (%)	3 mo. n (%)	6 mo. n (%)	9 mo. n (%)	12 mo. n (%)
	42	40	39	38	37
0	27 (64)	21 (53)	20 (51)	22 (58)	12 (32)
1	1 (2)	0	0	0	1 (3)
2	0	0	0	0	0
3	10 (24)	10 (25)	9 (23)	6 (16)	11 (31)
4	0	0	0	1 (3)	1 (3)
5	4 (10)	9 (22)	10 (26)	9 (24)	12 (32)

n=number

Table 3. Serum biochemical indices in dogs with biliary sludge over 12-month period

Variable	0 mo.	3 mo.	6 mo.	9 mo.	12 mo.	P value
n	42	42	42	41	38	
ALT (U/L)*	38 (14-450)	40 (14-367)	36 (16-141)	42 (15-172)	41.5 (17-131)	0.14
ALP (U/L)*	29 (6-225)	30.5 (7-215)	30 (6-164)	30 (9-348)	27(11-103)	0.58
GGT (U/L)*	3.5 (0-18)	3 (0-11)	3 (0-8)	3 (0-12)	3 (0-7)	0.48
Tbili(mg/dL)*	0.2 (0.1-0.5)	0.2 (0.1-0.5)	**0.2 (0.1-0.4)**	**0.2 (0.1-0.4)**	**0.2 (0.1-0.3)**	**0.0026§**
Trig (mg/dL)*	46 (24-493)	52 (24-84)	51 (30-135)	53 (33-101)	53.5 (23-170)	0.148
Chol (mg/dL)**	207 (±53.2)	207 (±53.5)	209 (±52.3)	204 (±47.5)	208 (±53.3)	0.70
TCa (mg/dL)**	9.69 (±0.49)	9.7 (±0.38)	9.8 (±0.51)	9.7 (±0.41)	9.8 (±0.21)	0.055
Alb (g/dL)*	3.3 (2.1-3.7)	**3.2 (2.2-3.7)**	3.25 (2.3-3.8)	**3.2 (2.2-3.7)**	3.2 (2.1 – 3.7)	**0.0055§**

*median (range), ** mean (standard deviation), § statistical significance, n=number

Table 4. Degree of sludge and GB measurements in dogs over 12-month period

Variable	0 mo.	3 mo.	6 mo.	9 mo.	12 mo.	*P*
n	42	42	42	41	38	
Relative Sludge**	27% (±20%)	28% (±18%)	29% (±20%)	30% (±17%)	33% (±21%)	0.45
Degree of Sludge *	1.5 (1-4)	1 (0-4)	1 (0-4)	2 (0-4)	2 (0-4)	*0.36*
GBV (mL/kg)**	1.03 (±0.59)	1.22(±0.74)	1.19 (±0.70)	1.18 (±0.88)	1.20 (±0.73)	0.19
GBV (mL)**	17.0 (± 12.2)	18.7 (±12.2)	18.7 (± 13.7)	17.4 (±13.8)	16.3 (±11.2)	0.54
GBW (mm)*	1 (0.44-1.53)	1 (0.66-1.62)	**1 (0.92–2.89)**	**1 (0.94-1.97)**	1 (0.90 – 1.35)	**0.015§**

*median (range), ** mean (standard deviation), § statistical significance, n=number

Table 5. Association of biochemical indices and GB measurements with spontaneous course of biliary sludge in dogs

Variable	Static	Increased	Decreased	Recurrent	*P*
n	17	12	8	4	
ALT (U/L)	47 (15-141)	40 (27-112)	46 (20-131)	29 (26-54)	0.36
ALP (U/L)	21 (11-100)	29 (13-107)	29 (17-103)	26 (14-78)	0.78
GGT (U/L)	4 (2-6)	2.5 (0-6)	4 (2-7)	3 (2-3)	0.56
Chol (mg/dL)	216 (135-359)	167 (144-266)	202 (167-263)	202 (156-223)	0.38
Trig (mg/dL)	53 (35-101)	48 (31-170)	76 (23-107)	58 (35-71)	0.47
Tbili (mg/dL)	0.2 (0.2-0.3)	0.2 (0.1-0.3)	0.2 (0.2-0.3)	0.2 (0.2-0.3)	0.16
TCa (mg/dL)	**9.9 (9.3-10.6)**	**9.5 (9.2-10.2)**	**10.2 (9.6-10.8)**	9.85 (9.4-10.0)	**0.024§**
Alb (g/dL)	3.3 (2.1—3.7)	3.0 (2.2-3.5)	3.3 (3.1-3.7)	3.3 (3.1-3.5)	0.12
GBV mL	15 (5.4-59)	9.7 (3.4-30)	14.5 (5.5-59.7)	14.1 (7.5-21.6)	0.38
GBW (mm)	1 (0.9 – 1.9)	1 (0.9-1.1)	1 (1-1.2)	1 (1-1.5)	0.39

median (range), § - statistical significance, n=number

Table 6. GB contents in dogs with development of non-dependent sludge over 12-month period

Dog	0 mo.	3 mo.	6 mo.	9 mo.	12 mo.
1	0	3	5	5	5
2	0	3	3	3	5
3	3	5	3	0	5
4	0	0	3	0	5
5	3	5	5	5	5
6	3	3	0	0	5
7	0	0	0	x	5
8	0	0	0	x	5
9	0	0	0	0	5
10	3	3	5	5	5

x– absence of sludge; 0 – dependent sludge; 3 – dependent and suspended sludge; 5- dependent, suspended, and non-dependent sludge. No dogs developed a score of 1 or 2.

Table 7. GB histopathology in dogs with or without biliary sludge

Gallbladder Histopathology	Biliary Sludge Group n	Control Group n
	27	4
Cystic Mucinous Hyperplasia	18	1
Mild	9	
Moderate	7	
Severe	2	1
Cholecystitis	10	2
Lymphoplasmacytic	9	2
Mixed	1	
Normal	7	2

n=number

Table 8. Liver histopathology in dogs with or without biliary sludge

Liver Histopathology	Biliary Sludge Group n	Control Group n
n	27	4
Steroid Induced Hepatopathy	15	1
Biliary Hyperplasia	5	1
Nodular Hyperplasia	2	
Hepatitis	3	
Reactive	2	
Lymphoplasmacytic	1	
Lymphoplasmacytic cholangitis	1	
Neoplasia	6	
Lymphoma	1	
Hepatocellular Carcinoma	2	
Histiocytic Sarcoma	1	
Metastatic	2	
Fibrosis	2	
Hepatocellular steatosis	2	2
Normal	3	2

n=number