Journal of Veterinary Internal Medicine
ACVIM
Open Access

J Vet Intern Med 2016;30:771–778

Spontaneous Course of Biliary Sludge Over 12 Months in Dogs with Ultrasonographically Identified Biliary Sludge

S.M. DeMonaco, D.C. Grant, M.M. Larson, D.L. Panciera, and M.S. Leib

Background: Biliary sludge is associated with gallbladder (GB) dysmotility and mucus hypersecretion suggesting a link between biliary sludge and the formation of GB mucoceles (GBM). If biliary sludge progresses to GBM, treatment to reduce the production and progression of sludge is warranted.

Hypothesis/Objectives: The objective of this study was to determine the course of biliary sludge in dogs.

Animals: Seventy-seven healthy, client-owned dogs ≥4 years of age screened for biliary sludge; 45 affected dogs identified.

Methods: Prospective, observational design. Serial ultrasound examinations were evaluated at 3, 6, 9, and 12 months to monitor degree of sludge based on proportion of 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, and GB dimensions.

Results: After 1 year of follow-up, the degree of sludge was mild (34%), moderate (47%), moderate to severe (13%), severe (3%), or absent (3%). There was no significant difference in median degree of sludge over 1 year (P = .36). There were no significant changes in the gravity dependency of sludge over 1 year. A subset of dogs, 24%, with initial gravity-dependent sludge developed a combination of nondependent and dependent sludge. Dogs had resolved (2%), decreased (19%), static (40%), increased (29%), or recurrent (10%) sludge at the conclusion of the study.

Conclusions and Clinical Importance: Biliary sludge was prevalent, affected dogs remained asymptomatic, and it rarely resolves in healthy dogs over a period of 1 year. Some dogs developed nongravity-dependent sludge within 1 year, which might indicate changes in consistency of sludge.

Key words: Gallbladder; Mucocele; Microlithiasis; Ultrasonography.

Sludge within the gallbladder (GB) is commonly seen during ultrasonographic examination and is defined as the presence of gravity-dependent, nonshadowing, echogenic material within the lumen of the GB.[1–3] The prevalence is not different among healthy dogs, dogs with hepatobiliary disease, or dogs with other diseases.[1] However, dogs with sludge have decreased GB motility when compared to dogs without biliary sludge, suggesting that biliary sludge might not be a benign process.[4]

Biliary stasis and modifications to bile within the GB promote the formation of biliary sludge,[5,6] and it is associated with mucus hypersecretion.[7,8] Hydrophobic bile acids stimulate mucus secretion from the GB epithelium.[9] Mucus accumulation within crypts and increased secretory vesicles developed within the GB epithelium of dogs with sludge and gallstones.[10–12] This suggests that prolonged exposure to concentrated bile, biliary sludge, or both might potentiate cystic mucinous hyperplasia (CMH). The pathogenesis of gallbladder mucocele (GBM) formation is unknown, but GBMs are associated with CMH and GB dysmotility suggesting a possible association with biliary sludge.[2,4,13,14] Dogs with biliary sludge were more likely to have hepatomegaly, cardiopathies, and systemic drug use,[4] and had higher plasma cholesterol concentrations than dogs without sludge.[15] Hyperlipidemia and endocrinopathies are associated with GBM[4,13,16–19] and are speculated to promote biliary sludge.[13] However, iatrogenic hypercortisolism for 3 months did not result in sludge formation.[20] Biliary sludge is prevalent in Poodles, Beagles, and Cocker Spaniels.[15] Although, a higher prevalence of sludge has not been found in other breeds predisposed to GBM, Cocker spaniels have an increased risk of GBM supporting the potential for sludge to mucocele.[2,14,18,19,21] Biliary stasis and GB dysmotility, mucus hypersecretion, biliary sludge formation, and CMH may represent a continuum with formation of GBM as the end stage of the disease process.

Dogs with GBMs can develop cholecystitis (bacterial or sterile), and GB necrosis and rupture, which are life-threatening and require emergent surgical

Abbreviations:

ALP	alkaline phosphatase
ALT	alanine aminotransferase
CMH	cystic mucinous hyperplasia
GB	gallbladder
GBM	gallbladder mucocele
GBV	gallbladder volume
GBV/kg	gallbladder volume per kilogram
GBW	gallbladder wall
GGT	gamma-glutamyltransferase

From the Departments of Small Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA (DeMonaco, Grant, Larson, Panciera, Leib).

This study was performed at the Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA.

Data from this study were presented in part as an abstract at the 2015 ACVIM Forum, Indianapolis, IN

Corresponding author: S.M. DeMonaco, Department of Small Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA 24061; e-mail: demonaco@vt.edu.

Submitted October 8, 2015; Revised January 7, 2016; Accepted February 23, 2016.

DOI: 10.1111/jvim.13929

intervention.[2,14,21] Thus, it is important to identify dogs with GB dysfunction earlier in the course of disease to avoid potentially serious complications. Studies evaluating the spontaneous course of biliary sludge in dogs are lacking. The presence of biliary sludge varied between 33–50% in six healthy dogs over 6 months, but changes in volume and character of sludge were not evaluated.[20] 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 important and treatments should be developed to reduce the production and progression of sludge formation.

The aim of this study was to determine the course of biliary sludge in apparently healthy dogs using serial ultrasonographic examinations during 1 year. Serial serum biochemistries were performed to evaluate changes in biochemical markers of cholestasis. We hypothesized that biliary sludge would increase over time and, in some dogs, become solid and immobile. We also hypothesized that dogs with progressive sludge would have increasing GB volumes and biochemical cholestasis.

Materials and Methods

Study Population

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. Institutional Animal Care and Use Committee approval and informed owner consent were obtained. An age of 4 years or older was chosen to increase the likelihood of identifying dogs with biliary sludge as it has a higher prevalence in older dogs.[1,15] Healthy was defined as the absence of illness within the preceding 3 months, lack of chronic drug administration, and an unremarkable physical examination. Dogs were not excluded if they received flea, tick, or heartworm preventative, supplements for osteoarthritis, or short duration (<4 weeks) of systemic drug administration during the study. Biliary sludge was defined as gravity dependent, echogenic material without acoustic shadowing within the GB. Forty-five (58.4%) dogs with biliary sludge were identified and evaluated every 3 months for 1 year. Three dogs with biliary sludge were ultimately excluded from final analyses because of chronic medication administration or the development of serious disease not associated with the biliary tract. Each evaluation included a physical examination, serum biochemistry, and hepatobiliary (liver, pancreas, GB) ultrasound. Dogs were fasted for at least 12 hours before ultrasound examination.

Ultrasound Analysis

Ultrasound examinations were performed or supervised by a board-certified radiologist using an ultrasound machine[a] equipped with a preset broad bandwidth operating frequency transducer (8–5 mHz microconvex). Transverse and longitudinal images of the GB were obtained with dogs in dorsal and/or lateral recumbency via subcostal and/or right-sided intercostal approaches. Gallbladder dimensions included length (L) obtained from longitudinal images and width (W) and depth (D) obtained from the transverse images. Gallbladder dimensions were determined from 3 transverse and 3 longitudinal images and the mean measurements were recorded. Gallbladder volumes (GBV) were calculated using the ellipsoid method (Volume = $0.53 \times L \times W \times D$).[22] Relative GBV was calculated by dividing the GBV by kilograms of body weight (GBV/kg).[23] Gallbladder wall (GBW) thickness was measured in a standard location of the body of the GB[1] and any abnormalities in the structure of the GB were documented. In dogs where the GBW was too thin to accurately measure, 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.[b] Relative sludge was calculated as follows: (sludge area/GB area) × 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%) (Fig 1). Gallbladder contents were categorized based on gravity dependency as: 0 = dependent sludge, 1 = dependent and nondependent sludge (sludge adhered to nondependent GB wall), 2 = nondependent sludge, 3 = dependent and suspended sludge, 4 = suspended sludge (sludge that is not adjacent to the GB wall), 5 = suspended, dependent, nondependent sludge (Fig 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 was compared at initial and final examinations to categorize dogs as having decreased, static, or increased biliary sludge.

Biochemical Analysis

Blood was collected from each dog after each examination, and activities of alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), and concentrations of total bilirubin, cholesterol, triglycerides, albumin, and total calcium measured. These variables were chosen to evaluate markers of hepatobiliary disease and hyperlipidemias, to evaluate serum biochemical variables that may affect bile constituents implicated in the formation of biliary sludge,[5] and maintain consistency with previous studies of biliary sludge in dogs.[1,4,15]

Statistical Analysis

Normal probability plots were used to evaluate data distribution. The mean and standard deviation were listed for normally distributed data, and the median and range were listed for skewed data. 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 differences in GB contents over time. Kruskal–Wallis test was performed to determine significant differences in biochemical indices, GBW thickness, and GBV among dogs with decreased, static, increased, or recurrent biliary sludge. Associations between variables and increased sludge were tested using the Wilcoxon rank-sum test (age) and the Fisher's exact test (breed, sex, structural liver abnormalities, presences of heart murmurs, use of flea, tick and heartworm preventatives, and use of systemic drugs). Particular flea/tick, heart worm, and systemic drugs that were used and evaluated included ivermectin, selamectin, fipronil, lufenuron, spinosad, imidacloprid, milbemycin oxime, antibiotics, nonsteroidal anti-inflammatories, and prednisone. All statistical analyses were performed using commercial software.[c] Significance was determined at $P < .05$.

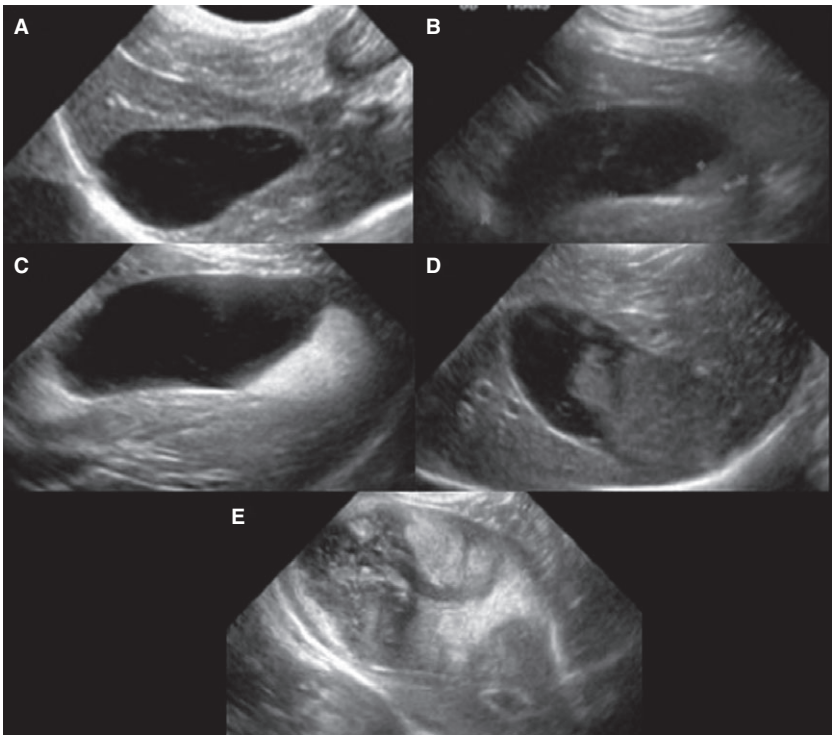

Fig 1. Representative ultrasound images of the gallbladder (GB) in dogs with (**A**) absence of biliary sludge—score 0, (**B**) mild biliary sludge—score 1, (**C**) moderate biliary sludge—score 2, (**D**) moderate to severe biliary sludge—score 3, (**E**) severe biliary sludge—score 4.

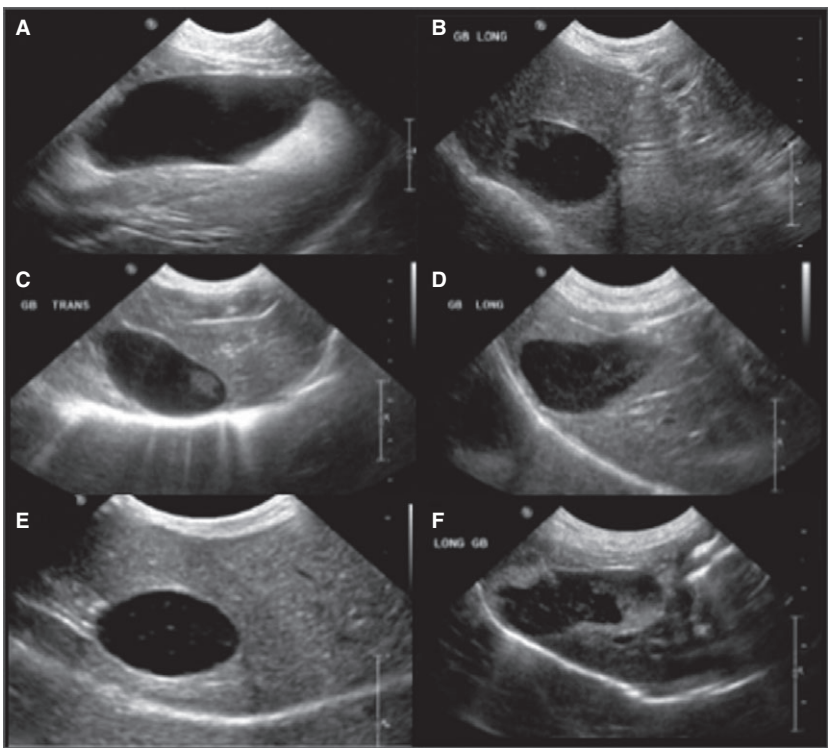

Fig 2. Representative ultrasound images of the gallbladder (GB) in dogs with (**A**) dependent biliary sludge only—GB content 0, (**B**) dependent and nondependent sludge—GB content 1, (**C**) nondependent sludge only—GB content 2, (**D**), dependent and suspended sludge —GB content 3, (**E**), suspended sludge only—GB content 4, and (**F**) suspended, dependent, and nondependent sludge—GB content 5.

Results

Population Data

Forty-two dogs with biliary sludge were evaluated every 3 months for 1 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. Four dogs did not complete the study because they were unavailable for scheduled re-examinations. The mean age of dogs with biliary sludge was 6.4 years (±2.4 years). Twenty dogs were neutered males, 21 dogs were spayed female, and 1 dog was an intact male. The most frequently represented breeds included mixed-breed (n = 21), Pit bull Terrier (n = 3), Dachshund (n = 3), Boston Terrier (n = 2), Boxer (n = 2), Great Dane (n = 2), and Chihuahua (n = 2). There was 1 dog of each of the following breeds: Jack Russell Terrier, Labrador Retriever, Cocker Spaniel, Toy Poodle, Australian Shepherd, Maltese, and Rottweiler. None of the dogs developed clinical signs attributable to hepatobiliary disease during the study period. Heart murmurs were detected in 16% (7/42) of dogs at either at initial examination (n = 2) or at a later examination (n = 5). Four of these dogs had an echocardiogram performed which showed early mitral-valve degeneration. Use of flea and tick preventative occurred in 86% (34/42) of dogs, and use of heartworm preventative occurred in 90% (38/42). Use of glucosamine/chondroitin or fish oil supplementation occurred in 10% (4/42) of dogs. Short or intermittent (<4 weeks) use of systemic drugs occurred in 24% (10/42) dogs. Drugs administered systemically included nonsteroidal anti-inflammatories (n = 4), prednisone (n = 2), antihistamines (n = 1), and antibiotics (n = 8).

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. 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. The median degree of biliary sludge did not change significantly over time (P = .36) (Table 1). When analyzed as a continuous variable, mean relative sludge was also not significantly different over time (P = .45) (Table 1).

Biliary sludge was persistent, resolved, and recurrent in 88% (37/42), 2% (1/42), and 10% (4/42) of dogs, respectively. Biliary sludge decreased, increased, or was static in 19% (8/42), 29%(12/42), and 40% (17/42) of dogs, respectively.

Dogs with increased degree of biliary sludge (12/42, 29%) were compared to dog without increased degree of biliary sludge (30/42, 71%). The median age was not significantly different between dogs with increased biliary sludge (5.5 years, range 4–10 years) and without increased biliary sludge (5.5 years, range 4–12 years) (P = .99). Prevalence of breed (P = 1) and sex (P = .73) were also not significantly different. There was no significant difference pertaining to presence of a heart murmur, presence of liver abnormalities as detected by ultrasound, use of flea/tick preventative, use of heartworm preventative, or use systemic medications (Table 2). In addition, there were no significant differences between dogs with or without increased biliary sludge in regards to use of imidacloprid, fipronil, lufenuron, spinosad, milbemycin oxime, selamectin, nonsteroidal anti-inflammatories, antibiotics, or prednisone (Table 2). Dogs with increased biliary sludge were 4.6 times more likely to be exposed to ivermectin (OR 4.6, 95% CI 1.1–19.5) (P = .04).

Gallbladder Content

The prevalence of 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% (1/37), 31% (11/37), 3% (1/37), and 32% (12/37) of dogs, respectively. Frequency of GB contents in each category did not change significantly over time (P = .25, data not shown). Only 2 dogs with initial nondependent sludge (GB content score 5) had gravity-dependent sludge (GB content score 0 or 3) at the end of the study. Three dogs with initial nondependent sludge (GB content of 1 or 5) continued to have similar GB contents at the end of the study. Ten dogs with an initial gravity-dependent sludge (GB content of 0 or 3) developed some form of nondependent sludge (GB content of 5) by the end of the study. Of the 10 dogs that developed nondependent sludge (GB content score of 5) at the end of the study, 1 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 nondependent sludge (GB content of 5) and the remaining 9 dogs continued to have a dependent biliary sludge (GB content of 0 or 3).

Gallbladder Measurements

Initial and 12-month mean GBV/kg were 1.03 ± 0.59 and 1.20 ± 0.73 mL/kg respectively (Table 1). The mean GBV/kg was not significantly different over time (P = .19). The mean GBV was also not significantly different over time (P = .54). Gallbladder wall was too thin to accurately measure 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 = .81) (Table 1). However, GBW thickness was significantly different (P = .015) when comparing initial examination to 6 and 9 months examinations (Table 1). GBV and GBW thickness were not significantly different among dogs with

Table 1. Summary of laboratory and GB variables in dogs with biliary sludge over time.

Variable	0 Month (n = 42)	3 Month (n = 42)	6 Month (n = 42)	9 Month (n = 41)	12 Month (n = 38)	*P*-Value
ALT (U/L)*	38 (14–450)	40 (14–367)	36 (16–141)	42 (15–172)	41.5 (17–131)	.14
ALP (U/L)*	29 (6–225)	30.5 (7–215)	30 (6–164)	30 (9–348)	27 (11–103)	.58
GGT (U/L)*	3.5 (0–18)	3 (0–11)	3 (0–8)	3 (0–12)	3 (0–7)	.48
Tbili (mg/dL)*	0.2 (0.1–0.5)[a,b,c]	0.2 (0.1–0.5)	0.2 (0.1–0.4)[a]	0.2 (0.1–0.4)[b]	0.2 (0.1–0.3)[c]	.0026
Trig (mg/dL)*	46 (24–493)	52 (24–84)	51 (30–135)	53 (33–101)	53.5 (23–170)	.15
Chol (mg/dL)**	207 (±53)	207 (±54)	209 (±52)	204 (±48)	208 (±53)	.70
tCa (mg/dL)**	9.7 (±0.5)	9.7 (±0.4)	9.8 (±0.5)	9.7 (±0.4)	9.8 (±0.2)	.055
Alb (g/dL)*	3.3 (2.1–3.7)[a,b]	3.2 (2.2–3.7)[a]	3.25 (2.3–3.8)	3.2 (2.2–3.7)[b]	3.2 (2.1–3.7)	.0055
Rel sludge (%)**	27 (±20)	28 (±18)	29 (±20)	30 (±17)	33 (±21)	.45
Deg sludge*	1.5 (1–4)	1 (0–4)	1 (0–4)	2 (0–4)	2 (0–4)	.36
GBV (mL/kg)**	1.03 (±0.59)	1.22 (±0.74)	1.19 (±0.70)	1.18 (±0.88)	1.20 (±0.73)	.19
GBV (mL)**	17.0 (± 12.2)	18.7 (±12.2)	18.7 (± 13.7)	17.4 (±13.8)	16.3 (±11.2)	.54
GBW (mm)*	1 (0.44–1.53)[a,b]	1 (0.66–1.62)	1 (0.92–2.89)[a]	1 (0.94–1.97)[b]	1 (0.90–1.35)	.015

ALT, alkaline aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; Tbili, total bilirubin; Trig, triglycerides; Chol, cholesterol; tCa, total calcium; Alb, albumin; Rel, relative sludge; Deg, degree of sludge; GBV/kg, gallbladder volume per kilogram; GB, gallbladder volume; GBW, gallbladder wall.

*Median (range), **Mean (standard deviation), n = number, $P < .05$ denotes statistical significance, significant differences are denoted by different superscript letters within columns.

Table 2. Prevalence of heart murmurs, liver abnormalities, and medication exposure in dogs with or without increased biliary sludge.

Variable	Increased Sludge (n = 12)	Other (n = 30)	*P*-Value
Heart murmur	2 (17)	5 (17)	1.00
Liver abnormalities	1 (8)	3 (10)	1.00
Systemic medications	3 (25)	7 (23)	1.00
Flea/tick	8 (67)	26 (86)	.20
Heartworm	10 (83)	28 (93)	.56
Ivermectin	8 (67)	9 (30)	.04
Imidacloprid	4 (33)	6 (20)	.43
Milbemycin oxime	3 (25)	12 (40)	.48
Selamectin	1 (8)	6 (20)	.65
Fipronil	1 (8)	8 (26)	.25
Lufenuron	2 (17)	4 (13)	1.00
Spinosad	1 (8)	9 (30)	.23
Antibiotics	2 (17)	6 (20)	1.00
NSAIDs	1 (8)	3 (10)	1.00
Prednisone	1 (8)	2 (7)	1.00

n = number; number (%); increased sludge—dogs with increased degree of biliary sludge; other—dogs with decreased, static, recurrent, or resolved biliary sludge; NSAIDs—nonsteroidal anti-inflammatories. $P < 0.05$ denotes statistical significance.

recurrent, persistent, decreased, or increased degree of biliary sludge (Table 3).

Biochemical Markers of Hepatobiliary Disease

There were no significant differences in serum activities of ALT, ALP, GGT, and concentration of total calcium, cholesterol, and triglycerides over time (Table 1). There was a significant difference in median concentration of total bilirubin ($P = .0026$) and albumin ($P = .0055$); however, the median values were still within reference range (Table 1). Only 1 dog had consistently low serum albumin concentrations. When comparing biochemical markers of hepatobiliary disease among dogs categorized as having recurrent, static, decreased or increased degree of biliary sludge, there was only a significant difference in median total calcium concentration. Dogs with increased degree of biliary sludge had lower median total calcium concentrations compared to dogs with decreased or static sludge ($P = .024$) (Table 3). The median total calcium concentrations for all groups were within reference range.

Ultrasonography of the Gallbladder, Liver, and Pancreas

There were no ultrasonographic abnormalities detected in the GB of dogs with biliary sludge. In addition, ultrasound did not identify any abnormalities within the pancreas. Ultrasonography of the liver revealed abnormalities in 4 dogs. These findings included rounded liver margins with mottled parenchyma (n = 1) and hyperechoic nodules (n = 3). The liver was normal in size in all dogs.

Discussion

Biliary sludge is currently regarded as an incidental finding with a high prevalence in healthy dogs.[1] The findings of this study corroborate that a high prevalence of biliary sludge (57%) occurs in healthy dogs ≥4 years. To the authors' knowledge, the spontaneous course of biliary sludge in individual dogs has not been systemically evaluated. This study found that biliary sludge persists in 88% of healthy dogs and did not significantly increase or become more organized. By the end of the study, 29% of dogs had more sludge filling their GB and 24% developed some form of nondependent sludge. The persistence of sludge suggests it has the potential to develop into or contribute to GB disease. This may be evident in the two subsets of dogs that had sludge progression, particularly if they were to be followed over a

Table 3. Association of biochemical indices and gallbladder measurements with spontaneous course of biliary sludge in dogs.

Variable	Static (n = 17)	Increased (n = 12)	Decreased (n = 8)	Recurrent (n = 4)	*P*-Value
ALT (U/L)	47 (15–141)	40 (27–112)	46 (20–131)	29 (26–54)	.36
ALP (U/L)	21 (11–100)	29 (13–107)	29 (17–103)	26 (14–78)	.78
GGT (U/L)	4 (2–6)	2.5 (0–6)	4 (2–7)	3 (2–3)	.55
Chol (mg/dL)	216 (135–359)	167 (144–266)	202 (167–263)	202 (156–223)	.38
Trig (mg/dL)	53 (35–101)	48 (31–170)	76 (23–107)	58 (35–71)	.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)	.16
tCa (mg/dL)	9.9[a] (9.3–10.6)	9.5[a,b] (9.2–10.2)	10.2[b] (9.6–10.8)	9.9 (9.4–10.0)	.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)	.12
GBV (mL)	15 (5.4–59)	9.7 (3.4–30)	14.5 (5.5–59.7)	14.1 (7.5–21.6)	.38
GBW (mm)	1 (0.9–1.9)	1 (0.9–1.1)	1 (1–1.2)	1 (1–1.5)	.39

ALT, alkaline aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; Tbili, total bilirubin; Trig, triglycerides; Chol, cholesterol; tCa, total calcium; Alb, albumin; GBV, gallbladder volume; GBW, gallbladder wall.

Median (range), n = number; $P < .05$ denotes statistical significance; significant differences are denoted by different superscript letters within columns. Determination of static, increased, decreased, and recurrent sludge is based on changes in degree of biliary sludge by comparing the degree of sludge at initial examination to the available last examination. The 1 dog with resolved biliary sludge was excluded from analysis.

longer duration. However, biliary sludge does not seem to develop into clinically apparent GB disease, at least over the course of 1 year, in healthy dogs.

The majority of dogs had persistent sludge and all remained asymptomatic. The degree of biliary sludge at each examination was not significantly different, but it increased in 29% of all dogs. The reason for this finding is uncertain but may be a reflection of cholestasis and/or poor GB motility in those dogs. These findings are in contrast to those in people with biliary sludge. A study following humans for 3 years found that biliary sludge resolved in 17.7%, and was recurrent 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.[3]

Risk factors for the progression of increased biliary sludge in dogs were not found with the exception of a modest association between the use of ivermectin and increased degree of biliary sludge. This finding should be interpreted with caution and does not indicate that ivermectin leads to increased biliary sludge in dogs. Ivermectin is a commonly used microflilaricide. It undergoes minimal hepatic metabolism and is excreted largely unchanged into the bile.[24] The mechanism of how ivermectin could lead to increased biliary sludge in dogs is unclear as the effects of ivermectin on the mucosal function of the GB epithelium, GB motility, or bile lithogenicity are unknown. Ivermectin has been shown to have anti-inflammatory properties and reduces mucus hypersecretion in the airways of mice making ivermectin induced mucus hypersecretion of the GB an unlikely mechanism.[25] It is doubtful that use of ivermectin is a risk factor for the progression of biliary sludge to GBM formation as use of ivermectin was not a risk factor for GBM formation.[19] Further studies would be necessary to evaluate the role of ivermectin in formation of progressive biliary sludge.

Increased biliary calcium (total and ionized) can lead to calcium salt precipitation and biliary sludge formation.[26,27] If serum total calcium is related to increased biliary sludge formation, it would be expected that total serum calcium would be higher in those dogs as a result of increased calcium excretion into the bile. In this study, dogs with increased biliary sludge had lower serum total calcium concentrations. Measurement of ionized calcium would be a better determination of serum calcium in these dogs. Further evaluation of serum ionized calcium concentrations, biliary calcium concentrations, and perhaps, diet and serum vitamin D concentrations in dogs with biliary sludge would be needed to conclude further on the importance of calcium with progressive biliary sludge.

Nondependent or organized sludge would be expected in dogs before development of a GBM and dogs with GB content of 1, 2, or 5 might represent dogs that are developing immobile sludge, organized sludge, or both. Despite that the majority of dogs had persistent biliary sludge, there were no significant changes in the frequency of GB contents over time. However, a subset of dogs, 24%, that initially had dependent biliary sludge developed a combination nondependent and dependent sludge by the end of 1 year. These dogs were not necessarily the same dogs with increased degree of biliary sludge. The development of nondependent biliary sludge might have been because of changes in viscosity of biliary sludge, potentially because of increased mucin,[28] affecting how rapidly biliary sludge moves away from the nondependent to the dependent GB wall. Studies evaluating the composition of biliary sludge in dogs could help elucidate whether increased mucin is a feature of nondependent biliary sludge.

Most biochemical indices and GB measurements were not significantly different overtime in dogs. The clinical importance of the significant changes in albumin, total bilirubin, and GBW thickness are unknown, but could become more apparent if followed over a longer period of time. There was a significant difference in median GBW thickness at 6 and 9 months compared to the GBW thickness at 0 months, but most of the dogs had

normal GBW thickness (<2 mm) at each examination. 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.[29] Exact wall measurements in some dogs were difficult because of the very thin nature of the wall, and were assumed to be <1 mm. This occurred in approximately half the dogs in this study at each examination period. Therefore, the significant difference in GBW thickness in dogs at 6 and 9 months may not be a reflection of variation in GBW thickness, but rather variability in accuracy of measuring GBW thickness with ultrasound. Regardless, the changes in GBW thickness were minimal and within normal limits, and GBW thickness was not significantly different at the 12-month examination, making changes at 6 and 9 of uncertain importance.

The mean GBV/kg in dogs at the end of this study was 1.2 mL/kg (±0.73 mL/kg), and a significant change in GBV/kg was not found. Typically, fasting GBVs are ≤1 mL/kg in normal dogs.[23] Further assessment of GB motility in dogs with GBV >1 mL/kg requires calculation of an ejection fraction where normal is considered to be ≥25%.[23] Dogs with biliary sludge and GBM had larger GBV and decreased GB emptying compared to controls in a previous study.[4] Whether the dogs in this 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 or GBM formation.

The 1 year duration of this study could be a limitation since it is possible that clinically important change in biliary sludge might take longer than 1 year to manifest. When dogs were separated into groups based on the progression of biliary sludge, the numbers of dogs in each group were small; thus, a larger study population might be necessary to detect differences in dogs with persistent or progressive biliary sludge versus dogs where biliary sludge resolves. Dogs were not rotated in position during ultrasound examinations to assess for mobility of sludge. This could have led to misidentification of nondependent or 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 examination. 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. In addition, 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.

Biliary sludge did not significantly increase or become immobile over time in healthy dogs over the period of 1 year. It is possible that dogs in which biliary sludge persists could go on to form GBM if other risk factors associated with GBM are present, such as breed, GB dysmotility, hyperlipidemias, or endocrinopathies.[4,13,16–19] It is also possible that the progression of biliary sludge to GBM is insidious, taking longer than 1 year to cause significant changes in the relative amount or gravity dependency of biliary sludge. When considering the presence of biliary sludge in healthy dogs over the course of 1 year, it does not appear to be associated with significant biochemical or ultrasonographic GB abnormalities. Increased percentage of biliary sludge occurred in 29% of dogs, and 24% of dogs went on to develop nongravity dependent GB contents. These 2 groups might represent dogs that develop GBM perhaps due to changes in bile composition or GB dysmotility, and 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. 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 and its potential relationship with GBM formation.

Acknowledgments

The authors thank Dr. Stephen Werre for statistical assistance. Partial financial support of this study was funded from the Savannah and Barry French Poodle Memorial Fund and the Tommy Thompson Professional Award.

Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

Footnotes

[a] Philips iU22; Philips Medical Systems, Bothell, WA.
[b] OsiriX version 6.0.2, http://www.osirix-viewer.com.
[c] SAS version 9.4 Cary, NC.

References

1. Bromel C, Barthez P, Leveille R, et al. Prevalence of gallbladder sludge in dogs as assessed by ultrasonography. Vet Radiol Ultrasound 1998;39:206–210.

2. Besso J, Wrigley R, Gliatto J, et al. Ultrasonographic appearance and clinical findigns in 14 dogs with gallbladder mucocele. Vet Radiol Ultrasound 2000;41:261–271.

3. Lee SP, Maher K, Nicholls J. Origin and fate of biliary sludge. Gastroenterology 1988;94:170–176.

4. Tsukagoshi T, Ohno K, Tsukamoto A, et al. Decreased gallbladder emptying in dogs with biliary sludge or gallbladder mucocele. Vet Radiol Ultrasound 2012;53:84–91.

5. Ko C, Sekijima J, Lee SP. Biliary sludge. Ann Intern Med 1999;130:301–311.

6. Jungst C, Kullak-Ublick GA, Jungst D. Gallstone disease: Microlithiasis and sludge. Best Pract Res Clin Gastroenterol 2006;20:1053–1062.

7. Lee S, Nicholls J. Nature and composition of biliary sludge. Gastroenterology 1985;90:677–686.

8. Bernhoft R, Pellegrini C, Broderick W, et al. Pigment sludge and stone formation in the acutely ligated dog gallbladder. Gastroenterology 1983;85:1166–1171.

9. Klinkspoor J, Kuver R, Savard C, et al. Model bile and bile salts accelerate mucin secretion by cultured dog gallbladder epithelial cells. Gastroenterology 1995;109:264–274.

10. Dawes L, Nahrwold D, Rege R. Reversal of pigment gallstone disease in a canine model. Arch Surg 1989;124:463–466.

11. Englert E, Harmon C, Freston J. Studies on the pathogenesis of diet-induced dog gallstones. Am J Dig Dis 1977;22:305–314.

12. Wales E, Englert E, Peric-Goilia L, et al. The spontaneous amorphous black pigment gallstones of the domestic dog. J Comp Pathol 1982;92:381–385.

13. Aguirre A, Center SA, Randolph J, et al. Gallbladder disease in Shetland Sheepdogs: 38 cases (1995–2005). J Am Vet Med Assoc 2007;231:79–88.

14. Pike F, Berg J, King N, et al. Gallbladder mucocele in dogs: 30 cases (2000–2002). J Am Vet Med Assoc 2004;224:1615–1622.

15. Secchi P, Poppl AG, Ilha A, et al. Prevalence, risk factors, and biochemical markers in dogs with ultrasound-diagnosed biliary sludge. Res Vet Sci 2012;93:1185–1189.

16. Mesich ML, Mayhew PD, Paek M, et al. Gall bladder mucoceles and their association with endocrinopathies in dogs: A retrospective case-control study. J Small Anim Pract 2009;50:630–635.

17. Walter R, Dunn M, d'Anjou M-A, et al. Nonsurgical resolution of gallbladder mucocele in two dogs. J Am Vet Med Assoc 2008;232:1688–1693.

18. Kutsunai M, Kanemoto H, Fukushima K, et al. The association between gall bladder mucoceles and hyperlipidaemia in dogs: A retrospective case control study. Vet J 2014;199:76–79.

19. Gookin JL, Correa MT, Peters A, et al. Association of gallbladder mucocele histologic diagnosis with selected drug use in dogs: A matched case-control study. J Vet Intern Med 2015;29:1464–1472.

20. Kook PH, Schellenberg S, Rentsch KM, et al. Effects of iatrogenic hypercortisolism on gallbladder sludge formation and biochemical bile constituents in dogs. Vet J 2012;191:225–230.

21. Worley D, Hottinger H, Lawrence H. Surgical management of gallbladder mucoceles in dogs: 22 cases (1999–2003). J Am Vet Med Assoc 2004;225:1418–1422.

22. Atalan G, Barr F, Holt P. Estimation of the volume of the gall bladder of 32 dog from linear ultrasonographic measurements. Vet Rec 2007;160:118–122.

23. Ramstedt K, Center S, Randolph J, et al. Changes in gallbaldder volume in healthy dogs after food was witheld for 12 hours followed by ingestion of a meal or a meal containing erythromycin. Am J Vet Res 2008;69:647–651.

24. Gonzalez Canga A, Sahagun Prieto AM, Jose Diez Liebana M, et al. The pharmacokinetics and metabolism of ivermectin in domestic animal species. Vet J 2009;179:25–37.

25. Yan S, Ci X, Chen N, et al. Anti-inflammatory effects of ivermectin in mouse model of allergic asthma. Inflamm Res 2011;60:589–596.

26. Dawes L, Nahrwold D, Rege R. Supersaturation of canine gallbladder bile with calcium bilirubinate during formation of pigment gallstones. Am J Surg 1989;157:82–88.

27. Dawes L, Nahrwold D, Rege R. Increased total and free ionized calcium in a canine model of pigment gallstones. Surgery 1988;104:86–90.

28. Jungst D, Niemeyer A, Muller I, et al. Mucin and phosphilipids determine viscosity of gallbladder bile in patients with gallstones. World J Gastroenterol 2001;7:203–207.

29. Nyland TG, Larson MM, Matoon JS. Liver. In: Nyland TG, Matoon JS, eds. Small Animal Diagnostic Ultrasound, 3rd ed. St. Louis, MO: Saunders; 2015:332–399.