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RESEARCH ARTICLE

Association Between Serum Total T4 Concentrations and Gallbladder Biliary Sludge in Dogs with Non-Thyroid Disorders: A Retrospective Case Control Study

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ABSTRACT

Biliary sludge (BS) is a collection of solid particles that have settled out of bile. It is composed of precipitated crystals, glycoproteins, calcium salts, cellular debris and mucins. It can cause complications like biliary colic, acute cholecystitis, and acute pancreatitis. Hypothyroidism has been linked to BS formation, but dogs with reduced serum total thyroxin (tT4) concentrations caused by non-thyroid diseases may be predisposed to BS formation. A retrospective study was planned to investigate BS stages and sludge scores in dogs with non-thyroid diseases and their association with serum tT4 concentrations. For this purpose, records of 68 dogs with non-thyroid diseases (45 dogs with BS and 23 without BS) from private owners were evaluated. Gravity-dependent biliary sludge (GBS), non-gravity dependent biliary sludge (NGBS), gallbladder mucocele (GBM), sludge score, serum concentrations of tT4 and bile acids were recorded in all dogs. Results showed that BS was present in 66.18% of the dogs, with GBS being the most common stage (84.44%), and 60.53% of GBS dogs had sludge score 1. The BS group had significantly lower mean serum tT4 ($P<0.05$) and higher mean bile acid concentrations ($P<0.01$) than the non-BS group. The proportion of dogs with lower tT4 and higher bile acid concentrations was higher in the BS group (65.9 and 37.8%, respectively) and was significantly associated with the presence of BS (tT4: $P<0.05$, OR=3.3; bile acid: $P<0.05$, OR=6.0). In conclusion, BS formation was prevalent with an over-representation of GBS score 1 in dogs with non-thyroid diseases. As seen in primary hypothyroidism, dogs with lower serum tT4 concentrations due to non-thyroid diseases and higher bile acid concentrations would be predisposed to BS formation.

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INTRODUCTION

Biliary sludge (BS) is a collection of solid particles that have settled out of bile. It is composed of precipitated crystals, glycoproteins, calcium salts, cellular debris and mucins. This sludge can cause complications like biliary colic, acute cholecystitis, and acute pancreatitis. It is identified on ultrasonography as a non-shadowing echogenic material within the gallbladder (Saunders *et al.*, 2017).

Biliary sludge is classified as gravity-dependent biliary sludge (GBS), non-gravity dependent biliary sludge (NGBS) and gallbladder mucocele (GBM). The GBM is the accumulation of bile in the gallbladder, which can result in pressure necrosis, biliary obstruction and peritonitis, if the gallbladder ultimately ruptures (Butler *et al.*, 2022). Both BS and GBM share a common pathogenesis, as both are composed of similar components. The progression of BS to GBM has been documented in previous reports (Mizutani *et al.*, 2017; Butler *et al.*, 2022). The gravity-dependent (mobile) form of BS (GBS) precedes the non-gravity-dependent (immobile) form (NGBS), which in turn precedes the development of GBM (Tsukagoshi *et al.*, 2012; Butler *et al.*, 2022). A semi-objective sludge scoring system ranging from 0 to 5 can be applied to assess the volume of BS within the gallbladder. A score of 0 is assigned if BS is not present in the gallbladder. Scores from 1 to 4 are assigned, depending on the percentage of gallbladder volume filled with BS at the GBS and NGBS stages. A

score of 5 is assigned if GBM formation was present. No score is assigned in cases of cholelithiasis (Butler *et al.*, 2022).

The association between hypothyroidism in dogs and the development of BS and GBM has been well-documented (Allerton *et al.*, 2018; Gookin *et al.*, 2018). The reduced circulating levels of thyroid hormones can affect liver metabolism and promote gallbladder abnormalities with a reduction in the bilirubin excretion rate, hypotension with delayed biliary emptying, mucus hypersecretion, and hypercholesterolemia, favoring the formation of BS and GBM (Kook *et al.*, 2012; DeMonaco *et al.*, 2016). A number of conditions have been associated with BS formation, including hyperadrenocorticism (Aicher *et al.*, 2019; Jaffey *et al.*, 2019), hyperlipidaemia (Kutsunai *et al.*, 2014; Jaffey *et al.*, 2019), hepatobiliary damage (DeMonaco *et al.*, 2016), diabetes mellitus (Jaffey *et al.*, 2019), cardiopathies (DeMonaco *et al.*, 2016), gastrointestinal disorders, neoplastic disorders, orthopaedic disorders, renal disorders (Smalle *et al.*, 2015; DeMonaco *et al.*, 2016), various systemic medications (Daminet and Ferguson, 2003), and certain breed predispositions (Aguirre *et al.*, 2007; DeMonaco *et al.*, 2016). It would be interesting to assess whether the reduction in serum total thyroxine (tT4) concentrations in these non-thyroid conditions plays a significant pathogenic role in the formation of BS.

The main objective of this study was to assess if dogs with lower blood tT4 concentrations due to non-thyroid diseases might be predisposed to BS formation, analogous to the predisposition observed in primary hypothyroidism. For this purpose, a retrospective analysis of the various stages of BS and gallbladder filling in dogs with non-thyroid diseases was carried out.

MATERIALS AND METHODS

Experimental animals: This retrospective study included records of dogs that had undergone abdominal ultrasound, a complete blood test for serum total T4 (tT4) determination, diagnosed with conditions other than hypothyroidism, and had not received any medication during the previous three weeks. Dogs with non-thyroid diseases but showed low serum tT4 (<1.5µg/dL) and elevated thyroid stimulating hormone (TSH; >0.6ng/mL) or in the upper half of the normal range (0.03-0.6ng/mL) were excluded because of the possibility of primary hypothyroidism. Furthermore, dogs from breeds predisposed to developing BS, such as Beagle, Cocker Spaniel and Poodle breeds (Bandyopadhyay *et al.*, 2007), Border terriers (Allerton *et al.*, 2018) and Shetland Sheepdogs (Aguirre *et al.*, 2007) were also excluded from the study.

The records of 2,040 dogs from private owners that were presented to the Internal Medicine Service in the Veterinary Clinical Hospital, University of Córdoba, Spain, over a one-year period from June 2023 to July 2024 were evaluated. A total of 68 dogs, 30 males and 38 females, age ranged 1.2-16.0 years and of different breeds that met the inclusion criteria were assigned to the BS (n=45) and non-BS (n=23) groups, depending upon ultrasound findings.

Abdominal ultrasound examination: All dogs were subjected to abdominal ultrasound examination, using a B-mode, real time ultrasound machine equipped with CA123 (6-10mHz) and LA5213 (7-13mHz) transducers (Esaote MyLab® 30 Vet Gold, Esaote, Barcelona, Spain). Two specialized sonographers blindly scored the ultrasound scan records, paying particular attention to the wall and intraluminal contents of the gallbladder in both the right and left lateral views. The presence of non-shadowing, echogenic material within the gallbladder lumen was considered as showing presence of BS (Lawrence and Steiner, 2017). The classification of BS was as follows (Tsukagoshi *et al.*, 2011): gravity-dependent or mobile BS (GBS), when sludge was free within the gallbladder lumen and changed its positions with gravity; non-gravity-dependent or immobile BS (NGBS), when sludge was adhered to the wall of the gallbladder; and gallbladder mucocele (GBM), when striated or stellate bile patterns were visible in the gallbladder lumen.

The sludge scoring system developed by Butler *et al.* (2022) was conducted on a scale from 0 to 5 to estimate the volume of BS within the gallbladder. A score of 0 was considered if no sludge was present. Depending on the percentage of gallbladder volume occupied by the sludge, scores were from 1 to 4: 1 for <25% sludge, 2 for 26-50% sludge, 3 for 51-75% sludge, and 4 for >75% sludge. A score of 5 was assigned if GBM formation was present. Cholelitiasis was not considered using this score system. Dogs were excluded from the study if there were discrepancies between the observations of two sonographers.

Serum biochemical analysis: Blood samples were obtained from the cephalic vein of each dog after a fasting time of 12 hours. Blood samples were allowed to clot, the serum was separated and stored at -20ºC until use. All serum determinations were performed using a biochemical analyzer (COBAS 6000 analyzer®, Roche Diagnostics, Indianapolis, USA). Data of serum concentrations of tT4, bile acids, triglycerides, total cholesterol, total bilirubin, glucose, total proteins, albumin and serum activities of ALT, AST, ALP and GGT enzymes were recorded in all experimental dogs. Serum TSH concentrations were determined on all dogs to rule out hypothyroidism and thereby ascertain their eligibility for inclusion in the study. Following confirmation of diagnosis, serum TSH results were not included in the study.

Statistical analysis: Statistical analysis was performed using Prism 10.0 for Windows® (GraphPad software, San Diego, California, USA). Normal distribution of data was assessed by a Kolmogorov-Smirnov test. The Grubbs' test for outliers was run on all data sets (https://www.graphpad.com/quickcalcs/Grubbs1.cfm). Mean age and serum concentrations were compared between dogs of two groups (BS and NBS), using a two-tailed Mann-Whitney U test for nonparametric data. Contingency analysis to evaluate association between categorical variables BS, sex and out-of-interval serum determinations was conducted by a Fisher's exact test with Odds ratio (OR) calculation. A probability value of $P \leq 0.05$ was considered significant. An OR value of 1.5-

3.5 was considered to have a low correlation, OR value of 3.5-9.0 was considered to have moderate correlation and OR value >9.1 was considered to show high correlation (Secchi *et al.*, 2012).

RESULTS

The results showed that biliary sludge was identified in 66.2% (45 out of 68) dogs with non-thyroid diseases. The most prevalent disorders were chronic enteropathy (33.3%), followed by liver diseases (26.7%). Less common disorders included mammary neoplasia (13.3%), genitourinary diseases (6.7%), leishmaniasis (4.4%), dermatological diseases (4.4%), renal diseases (4.4%), cardiac diseases (4.4%) and pancreatitis (2.2%). In the non-BS group (33.8%; 23 out of 68 dogs), the most prevalent diseases were chronic enteropathy (34.8%) and liver disease (26.1%), with mammary neoplasia (13.0%), urogenital diseases (8.7%), cardiac diseases (8.7%), leishmaniasis (4.3%) and pancreatitis (4.3%) were present in low frequencies.

Sex distribution was 19(42.22%) males and 26(57.78%) females in the BS group, and 11(47.83%) males and 12(52.17%) females in the non-BS group. A Fisher's exact test showed non-significant association between sex and presence of BS (P=0.7; OR=0.79; 95% CI=0.29-2.20).

In the BS group, the age range was 1.7 to 16.0 years, with a mean age of 9.4±3.4 years, whereas in the non-BS group, the age range was 1.2 to 13.0 years, with a mean age of 8.0±3.0 years. Mean ages of both groups were non-significantly different (Mann-Whitney U test; P=0.117).

The BS group included 17 different breeds; apart from Crossbreds (n=16; 35.55%), the most common breeds were Yorkshire terrier (n=5; 11.11%), Wine Cellar Rat Hunter (n=5; 11.11%), Chihuahua (n=3; 6.67%) and Greyhound (n=3; 6.67%), with the remaining 13(28.89%) dogs being from the less common breeds. The non-BS group included 13 different breeds, the most common being Crossbreds (n=4; 17.39%), followed by Yorkshire terrier, Maltese and Greyhound (n=3; 13.04% each) and the remaining dogs (10; 43.48%) belonged to less common breeds.

In the BS group, majority of dogs showed GBS (84.44%), followed by NGBS, and GBM, while cholelithiasis was seen in only 2.22% dogs (Fig. 1). According to the sludge scoring system (Fig. 2), the majority of dogs in the GBS group had a score of 1 (23 of 38; 60.53%); with the proportion decreasing progressively to a score of 2, 3 and 4 (Fig. 2). Among four dogs in the NGBS group, two dogs (50.0%) showed score of 1 and one dog each (25%) had score of 2 and 3. Both of the dogs with GBM received a score of 5 (Fig. 2). The score system could not be applied to the one dog with Cholelitiasis.

The representative ultrasound images of different stages of biliary sludge (BS) within the gallbladder in dogs are shown in Fig. 3. Gravity-dependent BS having score of 1 (<25% sludge) is shown in Fig. 3A. Non-gravity dependent BS with score of 1 is shown in Fig. 3B. Fig. 3C shows the non-gravity-dependent BS having a score of 3 (51-75% sludge), Fig. 3D represents gallbladder mucocele (score 5); and colelitiasis is shown in Fig. 3E.

The mean serum tT4 concentration was significantly lower (P<0.05; Mann-Whitney U test) in the BS group than in the non-BS group (Table 1). Two dogs, one from each group, were considered outliers and excluded from the analysis of tT4 concentrations. The proportion of dogs with serum tT4 concentrations below the laboratory reference range (1.5-5.0µg/dL) was higher in the BS group than in the non-BS group (Fig. 4A). Contingency analysis (Fisher's exact test with OR calculation) showed a significant association between low tT4 and the presence of BS (P<0.05, OR=3.3 CI=1.2-9.9).

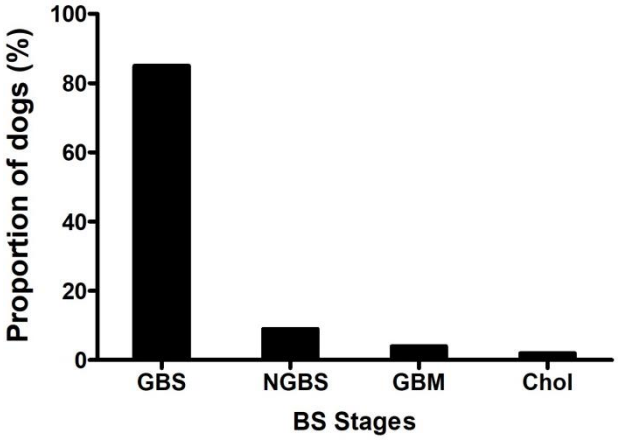

Fig. 1: Proportion of dogs in each stage of the biliary sludge (BS). GBS (gravity-dependent BS), NGBS (non-gravity-dependent BS), GBM (gallbladder mucocele), Chol (cholelitiasis).

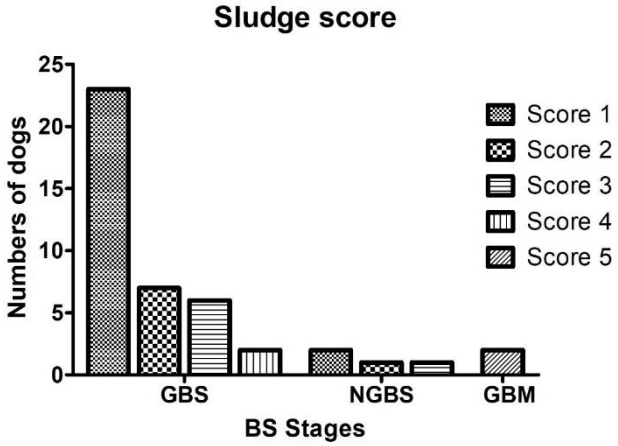

Fig. 2: Distributions of sludge scores in dogs of each stage of the biliary sludge (BS). GBS (gravity-dependent BS), NGBS (non-gravity-dependent BS), GBM (gallbladder mucocele).

Table 1: Descriptive statistics (mean±SD) for serum biochemical variables after excluding outliers in dogs with non-thyroidal diseases without and with gallbladder biliary sludge (BS)

Parameter	Without BS	With BS	P-value
tT4 (µg/dL)	1.7±0.6 (n=22)	1.4±0.8 (n=44)	0.03*
Triglycerides (mg/dL)	99.6±88.8 (n=23)	97.3±74.7 (n=44)	0.9 NS
Cholesterol (mg/dL)	227.9±69.0 (n=23)	244.2±96.9 (n=45)	0.7 NS
Bilirubin (mg/dL)	0.2±0.1(n=22)	0.3±0.2 (n=44)	0.08 NS
Glucose (mg/dL)	106.0±13.5 (n=23)	106.4±28.0 (n=45)	0.7 NS
Total proteins (g/dL)	6.6±0.9 (n=23)	6.6±0.9 (n=45)	0.9 NS
Albumin (g/dL)	3.2±0.5 (n=23)	3.0±0.6 (n=45)	0.1 NS
Bile acids (µmol/dL)	2.2±3.0 (n=22)	14.0±22.3 (n=44)	0.0004***
ALT (IU/L)	64.2±37.8 (n=22)	106.3±85.7 (n=44)	0.1 NS
AST (IU/L)	33.7±8.6 (n=23)	51.9±40.3 (n=42)	0.2 NS
ALP (IU/L)	93.0±52.1 (n=22)	207.6±344.7 (n=44)	0.2 NS
GGT (IU/L)	5.0±2.1 (n=22)	7.6±7.3 (n=44)	0.4 NS

NS: Non-significant difference; *significant differences (P<0.05); ***significant differences (P<0.01); n: number of dogs.

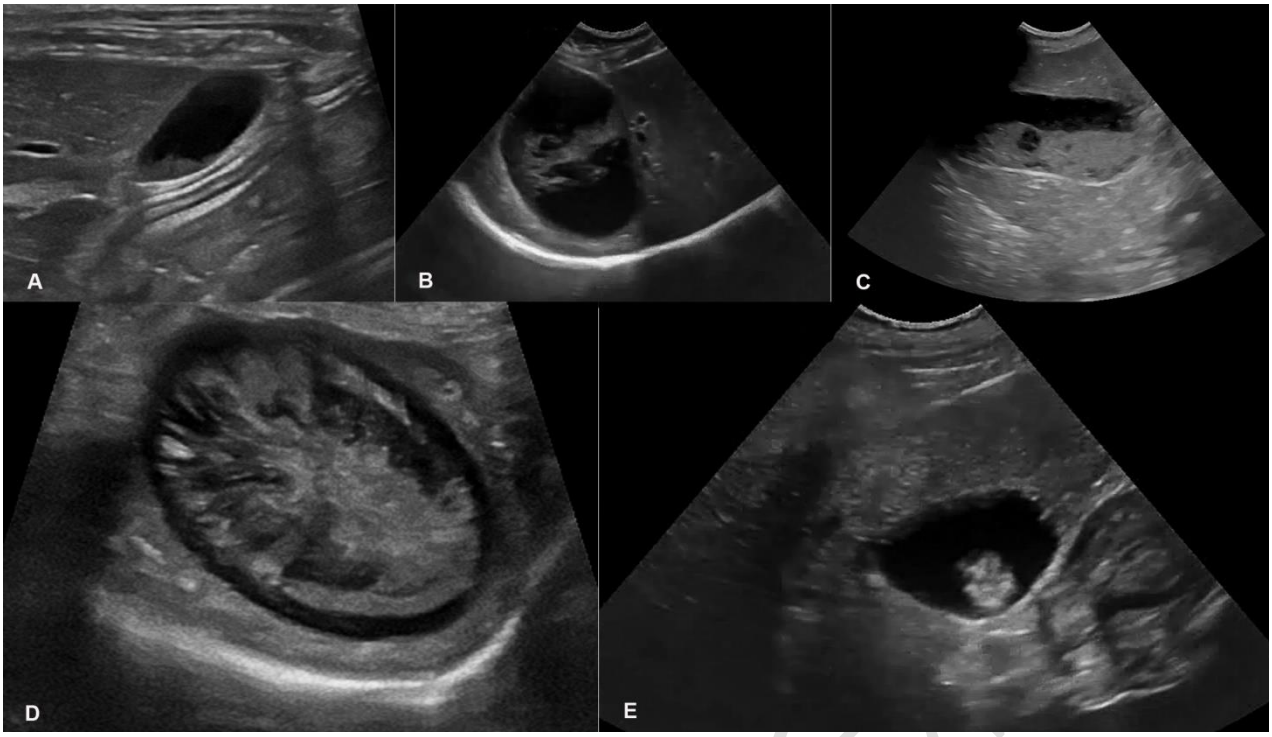

Fig. 3: Representative ultrasound images of the biliary sludge (BS) within the gallbladder in dogs: (A) gravity-dependent BS with score of 1 (<25% sludge); (B) non-gravity-dependent BS having score of 1 (<25% sludge); (C) non-gravity-dependent BS with score of 3 (51-75% sludge); (D) gallbladder mucocele (score 5); and (E) cholelitiasis.

Among different BS stages, the mean serum tT4 concentration was 1.5±0.9 µg/dL (range: 0.3-3.8) in the GBS group, 1.1±0.08µg/dL (range: 1.0-1.2) in the NGBS group, 0.93±0.1µg/dL (range: 0.8-1.0) in the GMB group and 0.9µg/dL in one dog with cholelithiasis. The proportion of dogs with serum tT4 concentrations below the reference range was low in the GBS group (56.8%) compared to 100% in NGBS, GBM and cholelithiasis groups (Fig. 4B). The small size of the groups precluded statistical analysis.

In the GBS group, the serum tT4 concentration was below the reference range in 60.9% in the sludge score of 1, 57.1% in score of 2, 50.0% in score of 3 and 100% in score of 4. All dogs in the NGBS (100%) and the cholelithiasis (100%) groups had lower tT4 concentrations.

The mean serum bile acid concentration was significantly higher (P<0.01; Mann-Whitney U test) in the BS group than in the non-BS group (Table 1). Two dogs, one from each group, were considered outliers and excluded from the analysis. The proportion of dogs with serum bile acid concentration above the laboratory reference range (0.0-14.9µmol/L) was higher in the BS group than in the non-BS group (Fig. 5A). There was a significant association (Fisher's exact test with OR calculation) between the proportion of dogs with serum bile acid concentrations above the laboratory reference range and the presence of BS (P<0.05, OR=6.0, CI=1.1-16.6).

Dogs with GBS had a lower median serum bile acid concentration of 4.3 (IQR 11.80-1.10) compared to dogs in the NGBS group (10.70, IQR 59.85-9.77) and GBM group (52.25, IQR 100.00-4.50). The one dog with cholelithiasis had a serum bile acid concentration of 73.1µmol/L. The proportion of dogs with serum bile acid concentration above the laboratory reference range was the highest (100%) in cholelithiasis and lowest (32.4%) in the GBS group (Fig. 5B). The small size of the groups precluded statistical analysis.

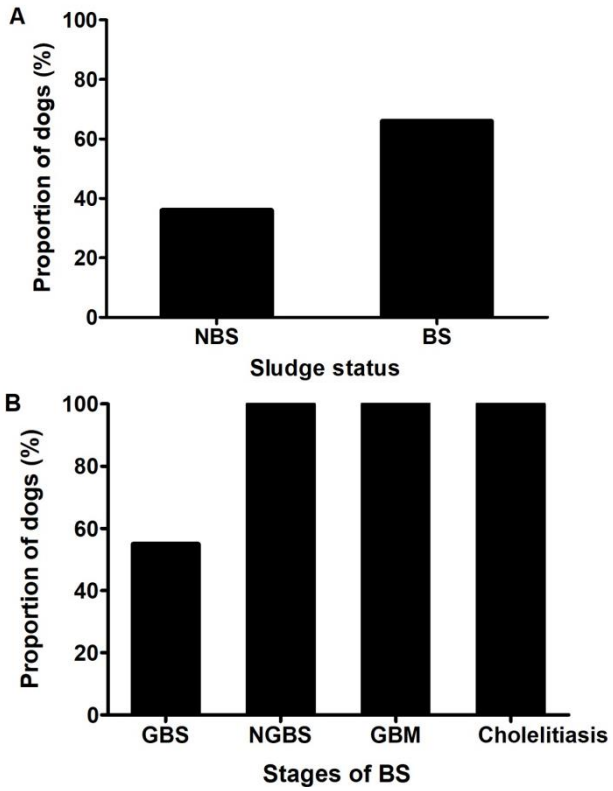

Fig. 4: Proportion of dogs with serum tT4 concentrations below the laboratory reference range (1.5-5.0µg/dL) in the non-biliary sludge (NBS) and in the biliary sludge (BS) groups (A). Proportion of dogs with serum tT4 concentrations below the laboratory reference range (1.5-5.0µg/dL) in different BS stages. GBS (gravity-dependent BS), NGBS (non-gravity-dependent BS), GBM (gallbladder mucocele) and cholelithiasis (B).

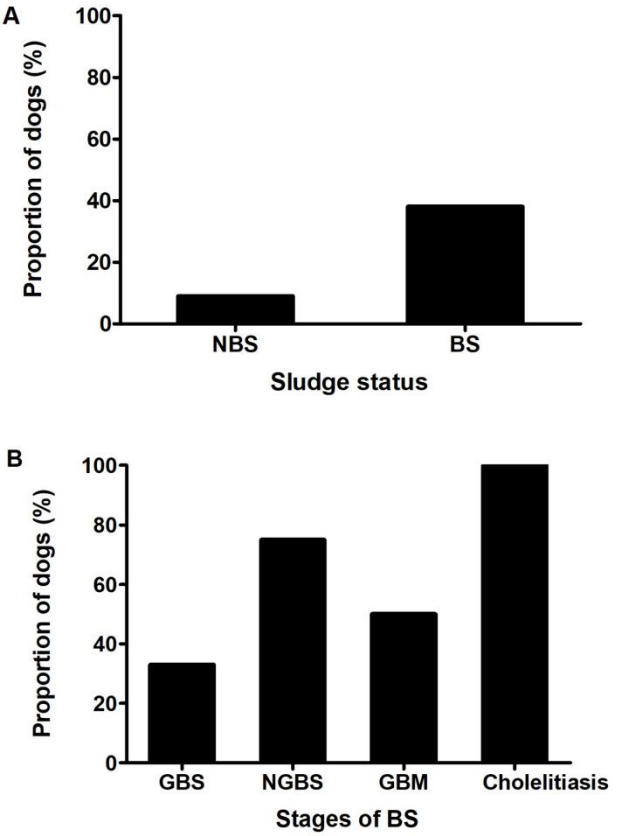

Fig. 5: Proportion of dogs with serum bile acid concentrations over the laboratory reference range (0.0-14.9µmol/L) in the non-biliary sludge (NBS) and biliary sludge (BS) groups (A). Proportion of dogs with serum bile acid concentrations over the laboratory reference (0.0-14.9µmol/L) in different BS stages. GBS (gravity-dependent BS), NGBS (non-gravity-dependent BS), GBM (gallbladder mucocele) and cholelitiasis (B).

According to the sludge score, in the GBS group, bile acid concentrations above the laboratory reference range were seen in 26.1% dogs with a score of 1, 57.1% dogs with a score of 2, 16.7% dogs with a score of 3 and 50% dogs with a score of 4. In the NGBS group, bile acid concentrations above the reference range were seen in 25% of dogs each with a score of 1, 2 and 3. This condition was also observed in 50% of dogs in the GBM group and in the only one dog with cholelithiasis (100%). The mean serum values for the remaining biochemical parameters analyzed (triglycerides, total cholesterol, total bilirubin, glucose, total proteins, albumin, and serum activities of ALT, AST, ALP and GGT enzymes) differed non-significantly between BS and non-BS groups (Table 1).

DISCUSSION

This retrospective study investigated whether dogs with low serum tT4 concentrations due to non-thyroid diseases were predisposed to BS formation, as has been described in dogs with hypothyroidism (Center, 2009; Mesich *et al.*, 2009; Gookin *et al.*, 2018). The study found that 66.18% of dogs with non-thyroid diseases had BS, which in most cases (60.53%) was GBS and with sludge score of 1. The results also showed that the mean serum tT4 was significantly lower in BS dogs than that in non-BS dogs, and the proportion of dogs with low tT4 was significantly higher, which supports the hypothesis that there is an association between low tT4 and the presence of BS in dogs with non-thyroid diseases.

Many clinical conditions have been associated with BS formation, yet it remains challenging to ascertain whether this association is causal or merely correlational, as might have occurred in our study. We found high prevalence of BS in dogs with non-thyroid diseases however, this finding could be associated with some specific diseases present in the animals studied. A significant proportion of non-thyroid diseases have been linked to low serum tT4 concentrations (Aicher *et al.*, 2019; Jaffey *et al.*, 2019; Rogers *et al.*, 2020; Bolton *et al.*, 2024). Consequently, a range of diseases were identified in the dogs included in the present study, yet their distribution remained comparable across the BS and non-BS groups. Chronic enteropathy and liver diseases exhibited comparable over-representation in both the BS and non-BS groups, attributable to their high clinical prevalence. Nevertheless, similar distribution of different diseases between the dogs of two groups does not necessarily substantiate a primary role of these conditions in the development of BS. This lack of association was further corroborated by Tsukagoshi *et al.* (2012), who demonstrated a comparable distribution of disorders in dogs with GBS and NGBS. Cardiopathies with hepatomegaly (Secchi *et al.*, 2012) and liver diseases (Butler *et al.*, 2022) were reported to be the most common diseases associated with the formation of BS and GBM in dogs, but again their distribution was almost similar in the two groups of dogs included in the present study. Consequently, the present findings indicate that the development of BS was associated with specific diseases, which also had their impact on thyroid hormones metabolism. Irrespective of the cause, decreased serum tT4 concentrations seems to be a contributing factor to the development of BS in dogs included in the present study.

Furthermore, it was observed that dogs with BS and non-thyroid diseases exhibited significantly higher serum bile acid concentrations compared to dogs without BS. Moreover, the proportion of BS dogs manifesting serum bile acid concentrations above the laboratory reference range values was higher than dogs without BS. The established correlation between BS formation and liver disease is a well-documented phenomenon. Most studies in the literature reported changes in some serum liver markers and lipid concentrations in dogs with BS (Secchi *et al.*, 2012; DeMonaco *et al.*, 2016) and with GBM (Tsukagoshi *et al.*, 2012); Jaffey *et al.*, 2019). A positive correlation between sludge score and serum GGT activity (Butler *et al.*, 2022) and higher serum total cholesterol and triglyceride concentrations in BS and GBM, respectively (Tsukagoshi *et al.*, 2012) have been reported. However, in the present study, non-significant differences were observed in serum triglycerides, total cholesterol, total bilirubin, glucose, total proteins, albumin concentrations, and serum activities of ALT, AST, ALP and GGT in dogs with or without BS, which is consistent with the findings reported by Secchi *et al.* (2012) and DeMonaco *et al.* (2016). Higher bile acid concentrations have been reported in various hepatic, digestive and endocrine disorders (Lawrence and Steiner, 2017; Tinted *et al.*, 2023). However, Tsukagoshi *et al.* (2012) reported non-significant changes in serum concentrations of bile acids in dogs with BS or GBM compared to healthy animals.

The significantly elevated (P<0.01) bile acid concentrations found in dogs with BS in our study could have been caused by the non-thyroid diseases and/or the reduction in serum tT4, or could simply be an effect of the BS formation. Further research is required to determine whether a reduction in serum bile acid concentration, whatever the underlying cause, would lead to a reduction or prevention of BS formation in dogs.

In the present study, most of the BS dogs were found to have GBS (84.44%), with less prevalent cases of NGBS (8.9%), GBM (4.44%), and cholelithiasis (2.22%). Comparable proportions were documented by Secchi *et al.* (2012), although in their study, GBM was relatively more prevalent than NGBS. The higher prevalence of GBS in our study could be explained by the fact that it is the earliest and less severe stage of BS formation, whereas more severe stages such as NGBS and GBM are less commonly seen (Mizutani *et al.*, 2017; Butler *et al.*, 2022). The progression of the condition from BS to GBM has been reported in only 13% dogs (Butler *et al.*, 2022) and cholelithiasis has been described as a rare condition with an overall prevalence of 0.97% (Ward *et al.*, 2020).

With regard to the sludge score, the majority of dogs in the GBS group (60.53%) had a sludge score of 1, with the proportion decreasing progressively to a sludge score of 2, 3 and 4. The progression of the problem from BS to GBM (score 5) occurs when sludge score increased from 1 to 4, with the highest score indicating a greater likelihood of developing GBM (Butler *et al.*, 2022). In the NGBS group, sludge scores exhibited variability; however, the limited sample size was the main constraint to establish the statistical significance of these observations. It is noteworthy that cholelithiasis has not been incorporated into the sludge score system (Butler *et al.*, 2022).

In 55.3% dogs of the GBS group, the serum tT4 concentration was below the reference range. A comparable proportion of dogs with low tT4 concentrations were identified in the sludge score category of 1, 2 and 3 (60.9, 57.1 and 50.0%, respectively). The only two dogs with a score of 4 in the GBS group, and all dogs in the NGBS, GBM and cholelithiasis groups, had lower tT4 concentrations. These findings suggest a potential association between lower tT4 concentrations and the severity of GBS, but not with the volume of gallbladder affected by sludge. In contrast to tT4 concentrations, higher bile acid concentrations did not appear to be associated with the severity of BS or the sludge score, as the dogs with a GBS score of 1-4 and those in the NGBS and GBM groups had similar bile acid concentrations profiles.

The main drawback of the present work is that the statistical analysis of the data could not be done among the different BS stages and sludge scores, as the numbers of dogs were very low in some groups. However, the findings suggest a potential association between lower tT4 concentrations and the more severe NGB and GBM stages. This association could be either causal or correlational, depending on whether both reflect the severity of a primary disease.

Conclusions: In dogs with non-thyroid diseases, the formation of BS was prevalent, with a significant over-representation of GBS score of 1. Mean serum tT4 level was significantly reduced, and the proportion of dogs with low tT4 was elevated in the cohort with BS, indicating that low tT4 was associated with the development of BS in dogs with non-thyroid diseases. Similarly, elevated serum bile acid levels were associated with the formation of BS. Moreover, no correlation between the sludge score and lower tT4 or higher bile acid serum concentrations could be noted. Further investigations with higher number of animals are necessary to determine the effect of thyroxine medication on dogs with BS, particularly in more severe cases.

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Authors contribution: RL conceived and designed the study, executed the experiment and analyzed the data. PJG conceived and designed the study and analyzed the data. MN analyzed the data. EMH executed the experiment. BB executed the experiment. All authors interpreted the data, critically reviewed the manuscript and approved the final version.

Ethical approval: The work described in this manuscript involved the use of non-experimental animals. Established and internationally recognised high standards of individual veterinary clinical patient care were always followed. Ethical approval from a committee was therefore not specifically required for this publication.

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