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Resting and postprandial serum bile acid concentrations in dogs with liver disease

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Abstract

Background: Serum bile acids (SBAs) are frequently measured in dogs. However, there is limited data comparing SBAs in different liver diseases diagnosed according to standardized histological criteria.

Objectives: To compare resting and postprandial SBAs, and determine their sensitivity and specificity, for various liver diseases in dogs.

Animals: Three hundred and forty-one client-owned dogs with suspected liver disease that had a liver biopsy and SBAs measured.

Methods: Multicenter retrospective study. Cases were classified according to standardized histological criteria. The sensitivity and specificity of resting and postprandial SBAs for the diagnosis of each liver disease, and all liver diseases combined, were calculated.

Results: The median resting SBAs were highest in dogs with cirrhosis (98.8 μmol/L; range, 6-135) and congenital circulatory anomalies (CCa; 79.45 μmol/L; 0.3-705). The highest median postprandial concentrations were found in CCa (126 μmol/L; 0-726) and chronic hepatitis (CH; 54.3 μmol/L; 0-260). Using the cut-off value of 10 μmol/L, the highest sensitivities of resting SBAs were recorded in dogs with CCa (87.5%; 95% confidence interval, 76.8-94.4) and CH (81.1%; 71.5-88.6). The sensitivities of postprandial SBAs were the highest in cholangitis (100%; 47.8-100.0) and CCa (91.1%; 78.8-97.5). The specificities of resting and postprandial SBAs for all diseases were 49.3% (37.6-61.1) and 29.7% (15.9-47.0), respectively.

Conclusions and Clinical Importance: Postprandial SBAs are more sensitive but less specific than resting SBAs for the diagnosis of liver disease. There were dogs in all categories of liver disease with resting SBAs <10 and >90 μmol/L. Therefore, careful interpretation of both normal and elevated values is required.

KEYWORDS

canine liver disease, cholestasis, hepatobiliary, WSAVA

1 | INTRODUCTION

Serum bile acids (SBAs) are increased in dogs for 3 reasons. First, if liver function is reduced there is a decreased clearance of bile acids from the portal circulation. Second, abnormal blood flow, such as occurs in a portosystemic shunt, results in bile acids bypassing the liver and therefore not being extracted by the hepatocytes. Finally, SBAs are increased when there is an impairment of excretion as a consequence of bile stasis.[1] Measurement of postprandial SBA concentrations is a more sensitive marker than resting SBAs concentration for the diagnosis of liver disease.[2]

In 2006 the World Small Animal Veterinary Association's (WSAVA) Liver Standardization Group produced a unified nomenclature for the histologic diagnosis of liver diseases in dogs.[3] These guidelines classify liver diseases morphologically into circulatory, biliary, parenchymal or neoplastic disorders. Circulatory disorders are divided into congenital circulatory anomalies (CCa) and disorders associated with outflow (hepatic congestion), or with portal hypertension. Within biliary tract diseases, there are subcategories for cholestasis and cholatestasis, biliary cystic disease and biliary atresia, cholangitis, and diseases of the gallbladder. Parenchymal disorders comprise reversible hepatocytic injury, which includes hepatocellular steatosis, steroid-induced hepatopathy and cloudy swelling, hepatic amyloidosis, hepatocellular death and inflammatory hepatopathies, hepatic abscesses and granulomas, hepatic metabolic storage diseases and miscellaneous disorders. Finally, the neoplastic group is composed of all benign and malignant primary liver neoplasms, and also nodular hyperplasia and metastatic neoplasia.[3]

Despite the fact that concentrations of SBAs are measured frequently in dogs, there is limited data on their ability to differentiate between different causes of liver disease. Moreover, little data exists to determine the sensitivity and specificity of SBAs for the diagnosis of individual diseases according to WSAVA histological criteria. Using non-standardized histological criteria, previous studies reported that resting and postprandial SBA concentrations had a sensitivity of greater than 65% for the diagnosis of different liver diseases.[2,4] In addition, resting and postprandial SBA concentrations greater than 30 μmol/L had specificities greater than 90% for the diagnosis of liver disease in dogs.[2,5]

The aims of this study were, therefore, (a) to compare resting and postprandial SBA concentrations in different liver diseases in dogs, and (b) to assess the sensitivity and specificity of these tests for different liver diseases classified according to the WSAVA classification system.

2 | MATERIALS AND METHODS

2.1 | Study design and case selection

Records of dogs which had liver tissues submitted for histological evaluation, and the concurrent measurement of SBA concentrations (within 7 days of histological biopsy), between September 2008 and September 2015 at 5 referral veterinary centers in the United Kingdom (Queen's Veterinary School Hospital, Pride Veterinary Centre, Anderson Moores Veterinary Specialists, Dick White Referrals and Davies Veterinary Specialists) were retrospectively reviewed. All histology samples were assessed by at least 1 board-certified pathologist. Details recorded included age, sex, concentrations of resting and postprandial SBAs, histological description, histological diagnosis and additional comments made by board-certified pathologists. Dogs that had a liver biopsy collected more than 1 week after measurement of SBAs were excluded.

Ethical approval was granted from the School of Veterinary Medicine and Science, University of Nottingham, UK, Clinical Ethical Review panel (reference 1610151103).

2.2 | Histological classification

The histological diagnosis was used to group cases according to WSAVA histological criteria for liver diseases in dogs using the 4 main morphological groups of vascular, biliary, parenchymal and neoplastic disorders.[3] Cases were classified into acute hepatitis (AH), cholangitis, chronic hepatitis (CH), CCa, cirrhosis, miscellaneous, neoplasia, nonspecific reactive hepatitis (RH), or reversible hepatocytic injury (RHI).

Dogs that had a histological diagnosis of cholangiohepatitis were placed into the cholangitis group unless there was evidence that the initial insult started in the hepatic parenchyma based on the comments by the histopathologist. Dogs that had histological features of more than 1 disease were placed into a single group which the pathologist stated to be the major disease.

Cases were excluded when the histological diagnosis was unclear, or when the diagnosis did not fit with WSAVA criteria. Dogs with obstructive cholelithiasis were also removed from the study, as the obstruction would have affected the SBAs concentrations, regardless of the histological changes present in the liver.

Dogs with different types of CCa, which included extrahepatic portosystemic shunt (EHPSS), intrahepatic portosystemic shunt

(IHPSS), primary portal vein hypoplasia (PPVH), and arteriovenous fistulas were classified into the CCa group as this was the histological diagnosis. The specific anomaly was recorded only when specified in the records.

Dogs with histological diagnosis of neoplasia were subclassified into focal neoplasia, diffuse neoplasia, or metastatic neoplasia. Cases with mesenchymal tumors, including hemangiosarcoma and poorly differentiated sarcomas, cases with hepatocellular neoplasia, including nodular hyperplasia, hepatocellular adenoma, hepatocellular carcinoma, and unspecified hepatocellular epithelial tumor, and cases with cholangiocellular tumors, including adenoma and carcinoma, were placed into the focal neoplasia group. Dogs with round cell tumors, including lymphoma, mast cell tumors, and unspecified type of round cell tumor, were classified into the diffuse neoplasia group. Dogs that had metastatic neuroendocrine tumors or metastatic adenocarcinomas were placed into the metastatic neoplasia group.

2.3 | Serum bile acids concentrations

Concentrations of SBAs were measured at 4 veterinary laboratories (staffed by board-certified clinical pathologists), and all using commercially available enzymatic spectrophotometric assays. The reagents used for the determination of the SBA concentrations at the different diagnostic laboratories were Randox (Randox Laboratories Ltd, Crumlin, United Kingdom), Dialab (DIALAB GmbH, Wiener Neudorf, Austria), and Sentinel (Sentinel Diagnostics, Milan, Italy). All 4 laboratories were members of an external quality control scheme and ran daily quality control assessments.

The type of sample (resting and/or postprandial) was recorded. The concentrations of resting and postprandial SBAs are presented as median and range unless otherwise stated.

2.4 | Statistical analysis

Age data are presented as median, and sex information is presented as the male to female (M : F) ratio for each liver disease.

Receiver operating characteristic (ROC) curve analysis (IBM SPSS Version 24.0) was used to calculate the sensitivity and specificity of resting and postprandial SBA concentrations for the diagnosis of each individual liver disease, and all liver diseases combined. Dogs with RH were used as the control group. Four different cut-off values (10, 30, 50, and 90 μmol/L) were evaluated. The 95% confidence interval (CI) was also determined for each result.

The area under the ROC curve (AUC) was used to determine the diagnostic performance of the tests for each disease. The diagnostic performance was classified as high (0.9 < AUC < 1), moderate (0.7 < AUC < 0.9), and low (0.5 < AUC < 0.7).[6] The null hypothesis for the ROC curve analysis is that the AUC is less or equal to 0.5.

The nonparametric Kruskal-Wallis test was used to evaluate if the distribution of SBA concentrations measured with the different reagents was statistically significant. The distribution of resting SBAs, measured with each of the reagents, in each of the individual groups with number of cases above 30 was also evaluated using the Kruskal-Wallis test.

3 | RESULTS

3.1 | Histopathology

Five dogs were excluded prior to analysis; 2 had nondiagnostic histological reports, 2 had a diagnosis which did not fit with the WSAVA criteria, and 1 had a cholelith causing extrahepatic biliary obstruction.

Three hundred forty-one dogs met the inclusion criteria and the histological diagnoses were as follows: AH (n = 12, 3.5%), cholangitis (n = 21, 6.2%), CH (n = 92, 27%), CCa (n = 64, 18.8%), cirrhosis (n = 4, 1.2%), miscellaneous (n = 4, 1.2%), neoplasia (n = 43, 12.6%), RH (n = 76, 22.3%), and RHI (n = 25, 7.3%).

Dogs that were categorized into the miscellaneous group had extramedullary hematopoiesis (n = 2) and hemosiderin accumulation (n = 2).

Those 64 dogs classified into the group of CCa were diagnosed with EHPSS (24/64, 37.5%), IHPSS (3/64, 4.7%), and PPVH (6/64, 9.4%). The type of vascular anomaly was not specified in the records in 31 of 64 (48.4%) cases.

Dogs that were diagnosed with neoplasia had focal neoplasia (n = 33), diffuse neoplasia (n = 6) and metastatic neoplasia (n = 4). Those dogs with focal neoplasia were diagnosed with hemangiosarcoma (n = 2), poorly differentiated sarcoma (n = 1), nodular hyperplasia (n = 3), hepatocellular adenoma (n = 6), hepatocellular carcinoma (n = 16), unspecified hepatocellular epithelial tumor (n = 3), cholangiocellular adenoma (n = 1), and cholangiocellular carcinoma (n = 1). Dogs with diffuse neoplasia were diagnosed with lymphoma (n = 4), mast cell tumor (n = 1), and unspecified round cell tumor (n = 1). Dogs with metastatic neoplasia were diagnosed with metastatic neuroendocrine tumors (n = 3) and metastatic adenocarcinoma (n = 1).

3.2 | Sex and age distribution

The distribution of sex and age among the different groups is detailed in Table 1. Of the total study sample, there were 195 males (57%) and 146 females (43%). Dogs with RHI had the highest M : F ratio (M : F ratio = 3.17), followed by RH (M : F ratio = 1.53), CCa (M : F ratio = 1.37), CH (M : F ratio = 1.24), neoplasia (M : F ratio = 1.05), cirrhosis (M : F ratio = 1), cholangitis (M : F ratio = 0.91), and AH (M : F ratio = 0.5).

The median age (years) for the different groups was distributed as follows: neoplasia (10), cholangitis (9), CH, cirrhosis, RH and RHI (7), and AH (4.96). Congenital circulatory anomalies were diagnosed more frequently in younger dogs, with a median age of 1.9 years.

3.3 | Serum bile acids

Resting SBA concentrations were available in 337/341 (98.8%) dogs, and the postprandial value was available in 151/341 (44.3%) cases.

The median and range concentrations of resting and postprandial SBA concentrations for each disease are displayed in Table 2. The median resting SBA concentrations was highest in cirrhosis

TABLE 1 Signalment of all dogs included in the study

	Number of dogs	Sex Female entire	Female neutered	Male entire	Male neutered	Sex ratio (male : female)	Median age in years (range)
Acute hepatitis	12	3	5	0	4	0.5	4.96 (0.25-11.17)
Cholangitis	21	2	9	1	9	0.91	9 (4.83-15)
Chronic hepatitis	92	9	32	10	41	1.24	7 (0.33-15)
Cirrhosis	4	0	2	1	1	1	7 (5–9)
Congenital circulatory anomaly	64	14	13	18	19	1.37	1.9 (0.33-8.17)
Miscellaneous	4	0	0	1	3	N/a	6.5 (5-9.83)
Neoplasia	43	1	20	6	16	1.05	10 (2.25-15.17)
Reactive hepatitis	76	8	22	15	31	1.53	7 (0.25-16)
Reversible hepatocytic injury	25	3	3	4	15	3.17	7 (0.33-14)

Notes: Age is expressed in years.

TABLE 2 Serum bile acid concentrations in dogs with different liver diseases

Resting serum bile acid concentrations disease groups (n)								
	AH (n = 12)	Cholangitis (n = 21)	CH (n = 90)	Cirrhosis (n = 4)	CCa (n = 64)	Miscell. (n = 4)	Neoplasia (n = 42)	RH (n = 75)	RHI (n = 25)
Median (range) (µmol/L)	24.55 (0-180)	48 (1-530)	41.85 (0-399)	98.8 (6-135)	79.45 (0.3–705)	8 (2–25)	11.7 (1-381)	10 (0.3-487)	15 (0-125)

Postprandial serum bile acid concentrations disease groups (n)								
	AH (n = 2)	Cholangitis (n = 5)	CH (n = 36)	Cirrhosis (n = 3)	CCa (n = 45)	Miscell. (n = 2)	Neoplasia (n = 11)	RH (n = 37)	RHI (n = 10)
Median (range) (µmol/L)	4.15 (4-4.3)	54 (27.4-160.1)	54.3 (0-260)	43 (7-230)	126 (0-726)	5.5 (0-11)	22 (5-868)	39 (0-382)	45 (0-212)

Abbreviations: AH, acute hepatitis; CCa, congenital circulatory anomaly; CH, chronic hepatitis; Miscell., miscellaneous; RH, nonspecific reactive hepatitis; RHI, reversible hepatocytic injury.

(98.8 µmol/L) followed by CCa (79.45 µmol/L), cholangitis (48 µmol/L), CH (41.85 µmol/L), AH (24.55 µmol/L), RHI (15 µmol/L), neoplasia (11.7 µmol/L), and RH (10 µmol/L).

The median postprandial SBA concentrations were highest in CCa (126 µmol/L) followed by CH (54.3 µmol/L), cholangitis (54 µmol/L), RHI (45 µmol/L) cirrhosis (43 µmol/L), RH (39 µmol/L), neoplasia (22 µmol/L), and AH (4.15 µmol/L).

Resting SBAs were available in 32 out of 33 dogs with focal neoplasia (median 9.5 µmol/L, range, 1-381 µmol/L), all 6 dogs with diffuse neoplasia (median 30.5 µmol/L, range, 9-49.3 µmol/L), and all 4 dogs with metastatic neoplasia (median 15 µmol/L, range, 1-30 µmol/L). Postprandial SBAs were available in 10 out of 33 dogs with focal neoplasia (median 21.35 µmol/L, range, 5-868 µmol/L), none of the dogs with diffuse neoplasia, and 1 dog out of 6 with metastatic neoplasia (52 µmol/L).

A bile acid stimulation test was performed in 147 dogs (43.1%). In 27.2% of the cases (40/147), the postprandial value was lower than the resting value. This was found in cholangitis (3 out of 5 dogs, 60.0%),

cirrhosis (1 out of 3, 33.3%), CH (11 out of 34, 32.4%), RH (9 out of 36, 25.0%), CCa (11 of 45, 24.4%), RHI (2 out of 10, 20.0%), and neoplasia (1 out of 10, 10.0%). Postprandial SBAs were lower than resting SBAs in both dogs with miscellaneous diseases, and in none of the 2 dogs with AH who had the bile acid stimulation test performed.

3.4 | Sensitivity, specificity, and diagnostic performance

The sensitivities of resting and postprandial SBA concentrations for each disease using the different cut-off values are shown in Table 3.

The highest sensitivities of resting SBAs using the cut-off value of 10 µmol/L were recorded in CCa (87.5%), cholangitis (85.7%), and CH (81.1%). When the cut-off values used were increased to 30, 50, and 90 µmol/L the highest sensitivities of resting SBAs were recorded in cirrhosis (75%, 75%, and 50%, respectively), CCa (70.3%, 64.1%, and 45.3%, respectively), and CH (56.7%, 41.1%, and 23.3% respectively).

TABLE 3 Sensitivity of resting and postprandial serum bile acids for the individual liver diseases using different cut-off values

Resting serum bile acid concentrations disease groups (n)

Cut-off value (μmol/L)	Acute hepatitis (n = 12) (95% CI)	Cholangitis (n = 21) (95% CI)	Chronic hepatitis (n = 90) (95% CI)	Congenital circulatory anomaly (n = 64) (95% CI)	Cirrhosis (n = 4) (95% CI)	Neoplasia (n = 42) (95% CI)	Reversible hepatocytic injury (n = 25) (95% CI)
10	58.3% (27.7-84.8)	85.7% (63.7-96.9)	81.1% (71.5-88.6)	87.5% (76.8-94.4)	75.0% (19.4-99.4)	54.8% (38.7-70.1)	64.0% (42.5-82.0)
30	41.7% (15.2-72.3)	66.7% (43.0-85.4)	56.7% (45.8-67.1)	70.3% (57.6-81.1)	75.0% (19.4-99.4)	16.7% (7.0-31.4)	16.0% (4.5-36.1)
50	25.0% (5.5-57.2)	38.1% (18.1-61.6)	41.1% (30.8-52.0)	64.1% (51.1-75.7)	75.0% (19.4-99.4)	9.5% (2.7-22.6)	16.0% (4.5-36.1)
90	16.7% (2.1-48.4)	23.8% (8.2-47.2)	23.3% (15.1-33.4)	45.3% (32.8-58.2)	50.0% (6.7-93.2)	7.1% (1.5-19.5)	8.0% (1.0-26.0)

Postprandial serum bile acid concentrations disease groups (n)

Cut-off value (μmol/L)	Acute hepatitis (n = 2) (95% CI)	Cholangitis (n = 5) (95% CI)	Chronic hepatitis (n = 36) (95% CI)	Congenital circulatory anomaly (n = 45) (95% CI)	Cirrhosis (n = 3) (95% CI)	Neoplasia (n = 11) (95% CI)	Reversible hepatocytic injury (n = 10) (95% CI)
10	0.0% (0.0-84.2)	100.0% (47.8-100.0)	75.0% (57.8-87.9)	91.1% (78.8-97.5)	66.7% (9.4-99.2)	72.7% (39.0-94.0)	70.0% (34.7-93.3)
30	0.0% (0.0-84.2)	60.0% (14.7-94.7)	55.6% (38.1-72.1)	86.7% (73.2-94.9)	66.7% (9.4-99.2)	27.3% (6.0-61.0)	60.0% (26.2-87.8)
50	0.0% (0.0-84.2)	60.0% (14.7-94.7)	52.8% (35.5-69.6)	80.0% (65.4-90.4)	33.3% (0.8-90.6)	27.3% (6.0-61.0)	50.0% (18.7-81.3)
90	0.0% (0.0-84.2)	40.0% (5.3-85.3)	36.1% (20.8-53.8)	68.9% (53.3-81.8)	33.3% (0.8-90.6)	9.1% (0.2-41.3)	30.0% (6.7-65.2)

Abbreviation: CI, confidence interval.

The highest sensitivities of postprandial SBAs using the cut-off value of 10 μmol/L were recorded in cholangitis (100%), CCa (91.1%), and CH (75.0%). When the cut-off values used were increased to 30 μmol/L, the highest sensitivities were seen in CCa (86.7%), cirrhosis (66.7%) and RHI and cholangitis (both 60.0%). Using the cut-off value of 50 μmol/L, the sensitivities of SBAs were higher in CCa (80.0%), cholangitis (60.0%), and CH (52.8%). Finally, the highest sensitivities using the cut-off value of 90 μmol/L were found in CCa (68.9%), cholangitis (40%), and cirrhosis (33.3%).

Combining all diseases together, the sensitivities of resting SBAs for the diagnosis of liver disease using the cut-off values of 10, 30, 50, and 90 μmol/L were 75.6% (95% CI 69.9-80.7), 49.2% (95% CI 43.0-55.5), 38.6% (95% CI 32.6-44.7), and 24.4% (95% CI 19.3-30.1), respectively. The sensitivities of postprandial SBAs using the same cut-off values were 81.6% (95% CI 73.2-88.2), 64.9% (95% CI 55.4-73.6), 59.6% (95% CI 50.0-68.7), and 44.7% (95% CI 35.4-54.3), respectively.

The specificities of resting SBAs for the diagnosis of liver disease using the cut-off values of 10, 30, 50 and 90 μmol/L were 49.3% (95% CI 37.6-61.1), 73.3% (95% CI 61.9-82.9), 82.7% (95% CI 72.2-90.4), and 94.7% (95% CI 86.9-98.5), respectively. The specificities of postprandial SBAs for the diagnosis of liver disease using the same cut-off values were 29.7% (95% CI 15.9-47.0), 43.2% (95% CI 27.1-60.5), 59.5% (95% CI 42.1-75.2), and 83.8% (95% CI 68.0-93.8), respectively.

The AUC demonstrated that the measurement of resting SBAs has a moderate diagnostic performance for the diagnosis of cirrhosis (0.80), CCa (0.80), CH (0.73), and cholangitis (0.75). Postprandial SBAs also showed moderate diagnostic performance for the diagnosis of CCa (0.80). The diagnostic performance of resting SBAs was low for the diagnosis of AH (0.63), RHI (0.55), and neoplasia (0.53). Postprandial SBAs showed low diagnostic performance for the diagnosis of cholangitis (0.66), cirrhosis (0.61), CH (0.58), and RHI (0.55) (Table 4).

The nonparametric Kruskal-Wallis test showed that the distribution of SBA concentrations was not significantly different across the 3 groups (reagents) ($P = .77$). The resting SBA concentrations, of all dogs included in the study, obtained with each of the reagents are displayed in Supporting Information Figure S1. The distribution of resting SBAs, measured with each of the reagents, in each of the individual groups with number of cases above 30 (CH, RH, CCa, and neoplasia) was also not significantly different ($P = .53$, $P = .07$, $P = .39$, and $P = 1.000$, respectively). The resting SBA concentrations of each of these individual groups, measured with the different reagents, are also displayed in Supporting Information Figures S2–S5.

4 | DISCUSSION

This multicenter retrospective study documents the concentrations of SBAs in liver diseases diagnosed according to WSAVA Liver Standardization Group. Moreover, this study to reports the sensitivity and specificity of resting and postprandial SBA concentrations in various liver diseases.

TABLE 4 Diagnostic performance of serum bile acids was determined using the area under the receiver operating characteristic curve

Histological diagnosis	n (resting SBAs)	AUC (95% CI)	n (postprandial SBAs)	AUC (95% CI)
Acute hepatitis	12	0.63 (0.46-0.81)	2	0.22 (0.08-0.35)
Cholangitis	21	0.75 (0.63-0.87)	5	0.66 (0.43-0.88)
Chronic hepatitis	90	0.73 (0.66-0.81)	36	0.582 (0.45-0.71)
Cirrhosis	4	0.80 (0.58-1)	3	0.61 (0.27-0.95)
Congenital circulatory anomaly	64	0.80 (0.72-0.87)	45	0.80 (0.70-0.89)
Neoplasia	42	0.53 (0.43-0.64)	11	0.46 (0.29-0.64)
Reversible hepatocytic injury	25	0.55 (0.43-0.68)	10	0.55 (0.33-0.77)

Notes: The diagnostic performance was classified as high (0.9 < AUC < 1), moderate (0.7 < AUC < 0.9), and low (0.5 < AUC < 0.7).
Abbreviations: AUC, area under the curve; CI, confidence interval; SBAs, serum bile acids.

This study documents overlap between SBAs, both resting and postprandial values, in different liver diseases in dogs, thereby limiting the utility of a single SBAs measurement as a test to differentiate between liver diseases. Resting SBAs below 10 μmol/L do not exclude liver disease being present, and moreover values above 90 μmol/L can be seen in dogs without liver disease. Furthermore, postprandial SBAs below 10 μmol/L occur in diseases in which elevated postprandial SBAs might be expected, such as CCa, CH, and cirrhosis.

In this study, the sensitivities of postprandial SBA concentrations for the diagnosis of liver disease were higher than resting SBAs using the different cut-off values. Resting SBAs had higher specificities than postprandial SBAs when the same cut-off values were used. Therefore, compared with resting SBAs, postprandial SBAs might be a more suitable screening test to exclude liver disease although, in our study, 18.4% of dogs with liver disease had postprandial values below 10 μmol/L and 35.1% below 30 μmol/L.

Using a cut-off value of 10 μmol/L, the sensitivity of resting SBA concentrations for the diagnosis of AH was 58.3%, which contrasts to 15% previously reported.[7] Using the same cut-off value, the sensitivities of resting and postprandial SBA concentrations for CH were 81.1% and 75%, respectively; however, these were below 60% when increasing the cut-off to 30 μmol/L. Similarly, concentrations of SBAs have been described as the most sensitive marker of CH, with sensitivities reported to be 61% to 89%, but values within the reference intervals can be found in early disease.[8] Damage of the hepatocytes might lead to reduced functional hepatic mass which impairs the clearance of bile acids from the portal blood.[1] It is therefore expected that dogs with AH, CH and cirrhosis can present with reduced function as well as intrahepatic cholestasis, both factors contributing to the increase in SBAs.[9] Depending on the degree of hepatocellular damage, the associated increase in SBAs can be variable, and we hypothesize that it is likely to be proportional to the amount of hepatocyte loss and the degree of cholestasis that occurs secondary to the hepatocellular damage.

In this study, resting SBAs were increased in 87.5%, 70.3%, and 64.1% of dogs with CCa using the cut-off values of 10, 30, and 50, respectively. These results are similar to those of previous studies which reported sensitivities of 87.1% to 98%, 70.5%, and 62.9%, respectively when using the same cut-off values.[10,11] The

abovementioned studies included dogs with EHPSS, IHPSS, PPVH and arteriovenous fistulas;[10] however, there were also some dogs included with acquired portosystemic shunts secondary to chronic hepatitis or cirrhosis.[11] Due to the different conditions classified as circulatory abnormalities in these studies, we acknowledge that the data from our study might not be directly comparable. In our study, we included 64 dogs with a histological diagnosis of CCa, but in 31 of these the specific type of vascular anomaly was not identified by the referral center. Consequently, we were not able to subclassify these cases further. It is therefore possible that some of the 31 dogs had arteriovenous fistulas, as well as PPVH or intrahepatic/extrahepatic shunts. In the group of dogs with CCa, the median concentrations of postprandial SBAs was the highest of all diseases (126 μmol/L), with 31 out of 45 dogs (68.9%) having SBAs above 90 μmol/L. In previous studies, the median values of postprandial SBAs were reported to be between 113 and 229.9 μmol/L.[2,12-14] 100% of 19 dogs with congenital portosystemic shunt (cPSS) have postprandial SBAs above 43 μmol/L, reflecting the high sensitivity of postprandial SBAs for the diagnosis of CCa.[13] In the aforementioned study, there was no crossover between healthy dogs and dogs with cPSS, suggesting postprandial SBAs could be used to rule out cPSS using a cut-off value of 30 μmol/L.[13] However, in our study we found that 4 out of 45 cases (8.9%) with CCa had postprandial SBAs below 10 μmol/L, 6 out of 45 cases (13.3%) had values below 30 μmol/L, and 9 dogs (20%) had values below 50 μmol/L. These results highlight that not all dogs with circulatory anomalies of the liver have increased postprandial SBAs. The maximum concentration of SBAs might not always occur 2 hours after a meal, as factors like responsiveness of the gallbladder to cholecystokinin or the intestinal transit time can affect this. Consequently, even though it is a sensitive test, 2-hour postprandial SBAs below 10 μmol/L can be found in dogs with CCa.

The term RHI is used to describe a group of hepatopathies that result from the reversible accumulation of water, glycogen, or fat in the cytoplasm of the hepatocytes.[15] This study documented that 4 out of 25 (16%) and 5 out of 10 (50%) dogs with RHI had resting and postprandial SBA concentrations above 50 μmol/L, respectively. These results contrast previous data, in which glucocorticoid hepatopathy, a type of RHI, was associated with normal to mildly increased SBAs, with an elevation of up 58 μmol/L in 1 study.[2]

Because the liver function in dogs with RHI is thought to remain unaffected, it is likely that the increase in SBAs is a consequence of bile stasis secondary to hepatocyte swelling.[16] The results of our study suggest that the degree of cholestasis associated with this disease should not be underestimated, and could be clinically significant. Moreover, there was considerable overlap between SBA concentrations of dogs with RHI and dogs with diseases of known clinical significance (ie, CCa, CH, and cholangitis). Thus, RHI should be considered a differential diagnosis in a dog with suspected liver disease presenting with high SBAs.

Cholangitis is a nonobstructive inflammatory disorder that affects the intrahepatic biliary ducts.[3,16] The inflammation is diffuse and targets the biliary tree, leading to intrahepatic cholestasis.[9] Concentrations of SBAs have not previously been evaluated in dogs with cholangitis. Three out of 21 dogs (14.3%) in the present study had resting SBAs below 10 μmol/L, but postprandial values were considerably higher, with all 5 dogs having values above 25 μmol/L. Interestingly, of the 5 dogs with cholangitis which had both resting and postprandial SBAs measured, none had a marked increase of SBAs in the postprandial sample compared to the resting sample. Three of those 5 dogs had lower postprandial SBA concentrations compared to the resting SBAs. The other 2 dogs had less than 2-fold increase of SBAs postprandially. We hypothesize that the lack of an increase in SBAs might be the result of an ineffective or slowed transportation of bile from the biliary system into the duodenum as a consequence of the inflammation of the biliary epithelium. Consequently, the pool of bile acids in the portal blood flow might not be significantly increased 2 hours after the ingestion of the meal, limiting the increase of concentrations of postprandial SBAs in the systemic circulation compared to the resting concentrations.

The specificities of SBAs for the diagnosis of liver disease were considerably lower than previously reported.[2,5] Using a cut-off value of 10 μmol/L, resting and postprandial SBAs had a specificity of 49.3% and 29.7%, respectively. The specificities increased to 82.7% and 59.5%, respectively when using the cut-off value of 50 μmol/L. This contrasts with previous studies, in which resting and postprandial SBA concentrations were reported to have a specificity of 100% using the cut-off values of 20 and 25 μmol/L, respectively.[2,5] The specificity of a test will vary depending on the cohort of dogs used in the control group. The control group used in our study comprised dogs with suspected liver disease and histological features consistent with RH, and this approach has been used in previous studies.[2,5] The authors of the present study conclude that the use of dogs with RH is the most optimal available option as those represent a sample of dogs that have clinical and clinicopathological characteristics similar to the diseased group, but histological changes suggestive of an extrahepatic cause. The clinical presentation of dogs with CCa is usually different than the presentation of dogs with RH, hence this might not be the optimal control for the CCa group. Ideally, a control group should consist of healthy dogs with no abnormalities on liver histology. However, it is very uncommon to identify such dogs as samples of liver tissues are usually taken because of clinical suspicion of liver disease, especially if the serum liver enzyme activities are increased. In addition, the use of

a control group comprised of dogs with suspected liver disease makes the specificities more relevant as they relate to the sample of dogs in which a liver biopsy could be taken, due to the clinical suspicion of liver disease.

Approximately one quarter (27.2%) of the dogs that underwent bile acid stimulation test had lower concentrations of postprandial SBAs than resting SBAs, which is similar to a previous report.[2] It has been suggested that this can be related to individual variations in gastric emptying, cholecystokinin response, intestinal transit time or intestinal absorption.[16,17] It could be that, in some cases, stimulation of the gallbladder contraction did not occur after the ingestion of the meal, or the peak of postprandial SBAs happened more than 2 hours after the release of cholecystokinin. This finding, however, is a possible limitation of the study, as the peak of postprandial SBAs of these dogs could have been missed and the obtained value could have been an underestimation.

The retrospective nature of this study means there were several additional limitations. We were unable to confirm if all patients had been starved sufficiently prior to measurement of resting SBA concentrations, which could have resulted in increased resting SBAs in the absence of liver disease. Unfortunately, another limitation was that we were not able to determine if the bile acid stimulation test was performed correctly, and it might be that some dogs did not eat an adequate amount or type of food to stimulate gallbladder contraction, thus affecting the concentrations of postprandial SBAs. Furthermore, SBA concentrations were measured in 4 different referral laboratories, using 3 different enzymatic spectrophotometric methods, which is likely to have increased the variability of our data; however, we would expect this limitation to decrease the calculated sensitivity and specificity values, therefore our data will still reflect minimum values for these parameters. Although the reference intervals reported by the different laboratories did vary, the vast majority of reference laboratories in the United Kingdom are unable to derive their own reference intervals for SBA concentrations due to lack of access to samples from a suitable number (>40) of healthy animals, because sampling of healthy animals cannot be performed in the United Kingdom on ethical grounds. Therefore many laboratories will adopt the reference intervals from other laboratories or from the literature, and they subsequently verify these intervals in their own laboratories using a smaller number of apparently healthy animals, to ensure that 95% of the values obtained in these animals fall within the adopted reference interval (reference interval transference).[18] The disadvantage of this approach is that the limits of the reference interval might be inaccurate, particularly given the variability that occurs between laboratories. Therefore, although comparison of the absolute SBA concentrations between different laboratories is not ideal, comparison of the bile acid concentrations normalized to the upper limit of the laboratory specific reference intervals would be unlikely to yield an improvement in the calculated sensitivity and specificity values. Moreover, many small animal veterinarians are using referral laboratories to measure SBA, and these laboratories could also use reference intervals adopted from the literature, or from other laboratories. Consequently, veterinarians will not be able to apply the values

normalized to the upper limit to their clinical practice. Furthermore, we anticipate that the biological variation of SBA in dogs is high compared to other biochemical analytes, as it has been shown in other species.[19,20] This can be due to diurnal rhythms of bile acid synthesis, spontaneous contraction of the gall bladder and variations in intestinal motility, among other factors. The biological variation of bile acids is likely to have significantly greater impact on the bile acid concentrations measured than the impact of the use of these different reagents. The Kruskal-Wallis test is a nonparametric analysis of variance that was used in our study to prove the hypothesis that the BAs measured with the 3 different reagents do not differ and are therefore directly comparable, allowing the combination of the SBA concentration values obtained with the 3 reagents, and the use of the different absolute cut-off values. In addition, most histological samples were only reviewed by 1 board certified pathologist, although they were all done so using published WSAVA criteria. However, we acknowledge that it is possible that some samples could have been misclassified. In addition, although the total number of cases in this study was very large, there were relatively small numbers of cases in certain individual diseases, thereby limiting conclusions that can be drawn. Further studies could aim to collect data from larger numbers of dogs with these diseases. Serum bilirubin concentrations, or the clinical sign of icterus, were not recorded by all centers, therefore these were not exclusion criteria. In our study, as the cases were from Specialist-lead referral centers, it is likely that dogs with icterus or elevated serum bilirubin did not have postprandial SBAs measured. However, SBAs are often included in routine biochemistry profiles, and consequently resting SBAs might have been measured, thus some dogs included might have had concurrent elevation of SBAs and bilirubin. Finally, all dogs in the study had suspected liver disease before the biopsy was taken, and for this reason, this could have resulted in the inclusion of dogs with more accentuated elevation of SBAs, not being a true representation of all dogs with liver disease.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by the School of Veterinary Medicine and Science, University of Nottingham, UK, Clinical Ethical Review panel (reference 1610 151 103).

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.