

# Gall bladder mucoceles and their association with endocrinopathies in dogs: a retrospective case-control study

**OBJECTIVE:** To investigate the relationship between endocrinopathies and diagnosis of gall bladder mucocele in dogs via a retrospective case-control study.

**METHODS:** Records of 78 dogs with a surgical or ultrasonographic diagnosis of gall bladder mucocele were examined for the presence or absence of hyperadrenocorticism, hypothyroidism and diabetes mellitus. Two age- and breed-matched controls for each gall bladder mucocele dog (156 total control dogs) were examined for the same concurrent diseases. A matched case-control analysis was performed using conditional logistic regression.

**RESULTS:** The odds of mucocele in dogs with hyperadrenocorticism were 29 times that of dogs without hyperadrenocorticism ( $P=0.001$ ; 95 per cent CI 3.8, 219.9). No difference was found between dogs with and without diabetes mellitus. Although a significant association was found between gall bladder mucocele and hypothyroidism, potential observation bias was also identified.

**CLINICAL SIGNIFICANCE:** Hyperadrenocorticoid dogs that were presented for acute illness with laboratory evidence of hepatobiliary disease should undergo evaluation for the presence of a biliary mucocele. Dogs diagnosed with a gall bladder mucocele should be screened for concurrent hyperadrenocorticism if clinical suspicion exists.

M. L. L. MESICH, P. D. MAYHEW\*,  
M. PAEK, D. E. HOLT AND D. C. BROWN

*Journal of Small Animal Practice* (2009)  
50, 630–635  
DOI: 10.1111/j.1748-5827.2009.00811.x  
Accepted: 8 June 2009

Department of Clinical Studies, Matthew J. Ryan Veterinary Hospital, University of Pennsylvania, 3900 Delancey Street, Philadelphia, PA 19104-6010, USA

M. L. L. Mesich's current address is Louisville Veterinary Speciality and Emergency Services, 13160 Magisterial Drive, Louisville, KY 40223, USA

\*P. D. Mayhew's current address is Columbia River Veterinary Specialists, 6818 NE 4th Plain Boulevard, Vancouver, WA 98661, USA

## INTRODUCTION

Canine gall bladder mucoceles (GBM) appear to be an increasingly frequent diagnosis in veterinary medicine (Besso and others 2000, Pike and others 2004a, Aguirre and others 2007). Although the definitions of GBM vary, the basic characteristics are an inappropriate accumulation of intraluminal, inspissated bile and/or mucus (Besso and others 2000, Mehler and others 2006). The gall bladder is often described as distended and containing a green-black gelatinous material or mass (Worley and others 2004). The inspissated

bile and mucus often extends throughout the biliary tree causing an extrahepatic biliary obstruction (Newell and others 1995, Pike and others 2004b). GBM have also been associated with gall bladder rupture (Worley and others 2004).

The presence of concurrent diseases in dogs with GBM has not been rigorously investigated. It has been suggested by multiple authors that endocrinopathies such as hyperadrenocorticism and hypothyroidism may play a role in gall bladder disease (Holt and others 2004, Aguirre and others 2007, Walter and others 2008). The goal of this study is to determine whether there is a significant association between the presence of endocrinopathies and the development of GBM in dogs. Our clinical impression before this study was that dogs with GBM are often concurrently hypothyroid or hyperadrenocorticoid but rarely, if ever, diabetic. We hypothesised that dogs diagnosed with hypothyroidism and hyperadrenocorticism would have greater odds for the diagnosis of GBM than dogs without those diseases. We hypothesised that there would be no relationship between the diagnosis of diabetes mellitus and the presence of GBM.

## MATERIALS AND METHODS

A retrospective case-control study was performed. Medical records from dogs that were presented to the Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania from January 1, 2000 to July 15, 2008 were reviewed to identify dogs with GBM and control dogs that did not suffer from the disease.

### Cases

GBM cases were identified in two ways:

- Ultrasonographic diagnosis of GBM by a board-certified radiologist according to criteria previously described (Besso and others 2000).
- Surgical diagnosis of GBM according to previously described criteria of gross

findings and/or histopathological findings by Besso and others (2000) and Pike and others (2004a).

### Controls

Incidence density sampling was used to identify two controls for each case. Each control was age- ( $\pm 2$  years) and breed-matched. When a case was an uncommon breed and an exact breed match was not available, a similar breed was selected. Mixed breed dogs were size-matched ( $\pm 10$  kg). Cases for which the diagnosis was made by abdominal ultrasound were matched with dogs that had a full abdominal ultrasound performed and the gall bladder was described as normal. Cases that were identified with surgery were matched with dogs that had undergone abdominal surgery in which no gall bladder abnormalities were identified. The sample size calculation was as follows: with 75 GBM cases, matching 1:2 cases with controls, assuming an event rate of 15 per cent in the cases (estimated based on recent case series by Pike and others (2004a) and Aguirre and others (2007) and clinical experience), an odds ratio of 3.0 would be detectable with  $\alpha$  0.05 and  $\beta$  0.80. From both cases and controls, the following information was extracted from the medical record: breed, age, sex, weight, steroid use, the presence of hypothyroidism, hyperadrenocorticism and diabetes mellitus. A dog was considered to be hypothyroid if a historical diagnosis was present or a thyroid panel was shown to be consistent with hypothyroidism within 6 months after presentation. All dogs diagnosed within 6 months after presentation with GBM or other presenting disease were diagnosed as hypothyroid by the internal medicine service. Various testing panels were done but no diagnosis was made on basis of a low total thyroxine (TT4) alone. All dogs had TT4 or free thyroxine (FT4) and thyroid stimulating hormone (TSH) or more extensive panel including various different combinations of free and total thyroid hormones and antibody to such hormones. Requirement for diagnosis was a low total or free T4 with elevated TSH and/or other supportive testing (total triiodothyronine, free triiodothyronine, antithyroxine antibodies, etc). A dog was

considered to have hyperadrenocorticism if a historical diagnosis was present or if either a low-dose dexamethasone suppression test or ACTH stimulation test was positive in combination with clinical signs within 6 months of presentation. A diagnosis of hyperadrenocorticism or hypothyroidism was not made if testing was performed during concurrent illness (that is, mucocele). A dog was considered to have diabetes mellitus if a historical diagnosis was present or persistent fasting hyperglycaemia with clinical signs within 3 months of presentation.

### Statistical analysis

Descriptive statistics were calculated. After confirming the non-normality of the data, continuous data were expressed as median values and ranges, and categorical data were expressed as frequencies for each group. A matched case-control analysis was performed. The univariate odds ratios and 95 per cent confidence intervals for GBM associated with hyperadrenocorticism, hypothyroidism and diabetes mellitus were estimated using conditional logistic regression for matched sets. Dog gender, bodyweight and steroid use were added to each model to determine whether they had a significant association with the outcome or were confounders (that is, changed the coefficient for the endocrinopathy by  $>15$  per cent). Fisher's exact tests were conducted to evaluate the differences within and between groups (GBM versus control) with regards to the (1) number of endocrinopathies diagnosed before versus immediately after GBM or control status designation, (2) number of dogs that underwent testing for endocrinopathies immediately after GBM or control status designation and (3) number of visits to the veterinarian made in the 6 months after GBM or control status designation. All statistical tests were two-sided. All analyses were performed using the STATA statistical package, version 10. P values less than 0.05 were considered as statistically significant.

### RESULTS

One hundred and forty-five medical records of dogs diagnosed with biliary or

gall bladder disease between January 1, 2000 and July 15, 2008 were examined. Seventy-eight of the dogs were diagnosed with GBM according to the inclusion criteria. There were 10 cases in which an exact breed match was unavailable and a similar breed was used, for example a Cairn terrier for an Australian terrier. Signalment characteristics of cases and controls identified from the same time period are presented in Table 1. Two dogs in the GBM group (3 per cent) and 4 dogs in the control group (3 per cent) were receiving steroids. Sixteen dogs in the GBM group (21 per cent) were diagnosed with hyperadrenocorticism, whereas 3 dogs in the control group (2 per cent) had that diagnosis. Eleven dogs in the GBM group (14 per cent) were diagnosed with hypothyroidism, whereas 8 dogs in the control group (5 per cent) had that diagnosis. Two dogs in the GBM group (3 per cent) and 3 dogs in the control group (2 per cent) were diagnosed with diabetes mellitus. The timing of the diagnosis of each endocrinopathy is detailed in Table 2. Significantly more dogs in the GBM group were diagnosed with hypothyroidism after the date of determination of GBM or control status than dogs in the control group. No significant difference was found in the timing of diagnosis of hyperadrenocorticism or diabetes mellitus between the control and the GBM group. The number of follow-up visits over 6 months between the control (median 1, mean 2.6) and the GBM (median 1, mean 2.4) group was not significantly different. Table 3 presents the total number of dogs in each group that underwent thyroid or adrenal function testing within 6 months after presentation. No significant difference was found between the total number of dogs tested for hyperadrenocorticism in the GBM group versus the control group. Six months after presentation, there were significantly more dogs tested for hypothyroidism in the GBM group compared with the control group.

Conditional logistic regression revealed that the odds of GBM in dogs with hypothyroidism were 3.0 times that of dogs without hypothyroidism ( $z=2.23$ ;  $P=0.026$ , 95 per cent CI 1.2, 7.7) and the odds of GBM in dogs with hyperadrenocorticism were

**Table 1. Signalment characteristics of dogs with gall bladder mucoceles compared with age- and breed-matched dogs without mucoceles that were presented between January 1, 2000 and July 15, 2008**

	Dogs with gall bladder mucocele (n=78)	Control dogs (n=156)
Age in years – median (range)	10.5 (3.0 to 17.0)	10.5 (1.9 to 17.0)
Bodyweight in kilograms – median (range)	11.7 (3.9 to 47.5)	12.9 (3.8 to 50.4)
Sex		
Females	40 (51 per cent)	91 (58 per cent)
Males	38 (49 per cent)	65 (42 per cent)
Breed		
Mixed breeds	16 (21 per cent)	33 (21 per cent)
Sheltie	10 (13 per cent)	19 (12 per cent)
Bichon	5 (6 per cent)	10 (6 per cent)
Dachshund	5 (6 per cent)	12 (8 per cent)
Beagle	4 (5 per cent)	4 (5 per cent)
Shih-tzu	4 (5 per cent)	8 (5 per cent)
Cocker spaniel	4 (5 per cent)	8 (5 per cent)
German shepherd	4 (5 per cent)	8 (5 per cent)
	Three or fewer of 21 other pure breed (34 per cent)	Six or fewer of 21 other pure breed (33 per cent)

**Table 2. Number of dogs diagnosed with an endocrinopathy before and after diagnosis of GBM or control status**

	HAC		HypoT		DM	
	GBM	Control	GBM	Control	GBM	Control
Endocrinopathy diagnosed <i>before</i> diagnosis of GBM or control status	7 of 16 (44 per cent)	2 of 3 (66 per cent)	6 of 11 (55 per cent)	8 of 8 (100 per cent)	1 of 2 (50 per cent)	2 of 3 (66 per cent)
Endocrinopathy diagnosed <i>within 6 months after</i> diagnosis of GBM or control status	9 of 16 (56 per cent)	1 of 3 (33 per cent)	5 of 11 (45 per cent)	0 of 8 (0 per cent)	1 of 2 (50 per cent)	1 of 3 (33 per cent)

GBM Gall bladder mucoceles, HAC Hyperadrenocorticism, HypoT Hypothyroidism, DM Diabetes mellitus

**Table 3. Total number of dogs in the GBM and control group tested for hyperadrenocorticism and hypothyroidism, regardless of result, within 6 months after presentation**

	GBM	Control
Tested for hyperadrenocorticism within 6 months after diagnosis of GBM or control status	14 of 78 (17.9 per cent)	15 of 156 (9.6 per cent)
Tested for hypothyroidism within 6 months after diagnosis of GBM or control status	6 of 78 (7.7 per cent)*	2 of 156 (1.3 per cent)*

GBM Gall bladder mucoceles  
\*Statistically significant difference P=0.05

29.0 times that of dogs without hyperadrenocorticism ( $z=3.26$ ;  $P=0.001$ ; 95 per cent CI 3.8, 219.9). Steroid use, bodyweight and gender were not significant

covariates in either model. There was no statistically significant association between the presence of GBM and the presence of diabetes mellitus.

## DISCUSSION

Multiple previous studies have suggested a possible association between gall bladder disease and select endocrinopathies (Holt and others 2004, Pike and others 2004a, Mehler and others 2006). A case series recently postulated a possible association between hypothyroidism and GBM formation (Walter and others 2008). Another study found that 31.5 per cent of Shetland sheepdogs with gall bladder disease (the majority having mucoceles) had elevated steroid hormone levels with 7.9 per cent having classical hyperadrenocorticism. This report also found that 13 per cent of these dogs had hypothyroidism (Aguirre and others 2007). Hyperadrenocorticism has also been confirmed in 23 per cent of 30 dogs with GBM in a separate study (Pike and others 2004a). This number is similar to the presence of 21 per cent found in our GBM population. Results of the current study show that a significant proportion of dogs diagnosed with GBM also have an endocrinopathy when compared with a matched control group. Hyperadrenocorticism was the endocrinopathy with the highest prevalence in this population of dogs with GBM. Hypothyroidism was also overrepresented in the diseased population but to a lesser degree than hyperadrenocorticism.

Although an association has been suggested, no clear role of hyperadrenocorticism in the pathogenesis of GBM has been proposed in dogs. To the authors' knowledge, no model of GBM exists in either dogs or other species. It is well known that immunosuppression is a feature of both hyperadrenocorticism and long-term steroid use (Reusch 2005). Resistance to bacterial infection may be decreased in these animals and predispose them to cholecystitis. In human beings and animals, cholecystitis has been associated with an increase in mucus production in the gall bladder (Sherlock 2002, Breitenstein and others 2006, Rege and Prystowsky 1996). A study conducted on the gall bladder mucosa of dogs has shown that inflammatory mediators such as prostaglandin 1 and 2 stimulate mucin secretion (Kuver and others 1994). Another study showed that two genes associated with the production of gel-forming mucins in the biliary

epithelial cells of mice were significantly overexpressed in response to lipopolysaccharide or TNF  $\alpha$  (Zen and others 2002). Although bacterial infection or inflammation may play a contributing role in GBM formation, it is unlikely that this is a key feature of pathogenesis as 20 to 55 per cent of GBM have no inflammation on histopathological examination (Amsellem and others 2006, Aguirre and others 2007). Bile or gall bladder samples from mucoceles only cultured bacteria in 9 to 35 per cent of cases in recent reports making lipopolysaccharide from bacterial infection an unlikely source of instigation (Pike and others 2004a, Worley and others 2004, Aguirre and others 2007).

Bile composition has been shown to change in people with hyperadrenocorticism and guinea pigs with long-term steroid administration (Yarimagan and Bor 1986, German and others 1989). Mucin secretion from epithelial cells of the canine gall bladder has been found to dramatically increase in response to increasing concentrations of bile acids, increasing contact time and to more hydrophobic bile acids (Klinkspoor and others 1995). This suggests that retention of bile in the gall bladder due to fasting, functional or physical obstruction may allow increased mucin production. It is currently unknown whether steroid use or overproduction changes specific bile acid ratios. If analogous bile changes occur in dogs, it may have relevance to gall bladder mucosal changes such as mucin hypersecretion in response to bile acids.

Changes in gall bladder motility have often been sited as a potential cause or contributor in GBM formation (Worley and others 2004). Dysmotility of the gall bladder via dynamic imaging studies has been documented before development of a mature mucocele in three dogs (Aguirre and others 2007). However, the severity and nature of disease in these gall bladders were not described and dysmotility could be a consequence of pathology rather than the cause. In people with hyperadrenocorticism, gall bladder dyskinesia occurred in 54 per cent of patients with hypermotility predominating. The presence of hypomotility tended to rise in people with prolonged endocrine disease (German and others 1989). This suggests that if similar

changes occur in Cushingoid dogs, gall bladder dyskinesia may not play a major role in GBM formation.

In dogs, selective inhibition of glucocorticoids caused a significant decrease in gall bladder motor function and that normal contractility was restored with the administration of hydrocortisone (Potapov and others 1976). No similar study in dogs looking at physiological effects of excess long-term steroids or spontaneous hyperadrenocorticism on gall bladder contractility is available. Other steroid hormones, specifically progesterone, testosterone and dihydrotestosterone, result in concentration-dependent inhibition of gall bladder motility in guinea pigs (Kline and Karpinski 2005, 2008). This may be true for hyperadrenocorticism resulting in a greater degree of effect on the gall bladder with larger steroid overproduction. In addition, other steroids and/or cortisol precursors may be excessively secreted in hyperadrenocorticism (Norman and others 1999, Benitah and others 2005). It is possible that overall gall bladder motility in hyperadrenocorticism is affected by a multitude of steroids and is the sum of their effects. The wide variation in gall bladder functional status in the Cushingoid human population studied by German and others (1989) lends support to this possibility.

In this study, hypothyroidism was overrepresented in the gall bladder mucocele population compared with the control group. Recent literature has investigated bile flow in hypothyroidism and found delayed emptying of bile into the intestine in both human beings and rats (Laukarinen and others 2002a, 2003). This appears to be a function of increased tonicity of the sphincter of Oddi rather than gall bladder changes per se, as there was no difference seen in the gall bladder contractility in euthyroid and hypothyroid people using nuclear cholecintigraphy (Laukarinen and others 2003). Thyroxine was found to allow relaxation of the sphincter of Oddi in pigs and human beings in the face of contraction stimulating substances. The absence of or low concentrations of thyroxine allowed a greater degree of contraction of the sphincter of Oddi (Laukarinen and others 2002b). Long-term decreased biliary emptying may cause

chronic gall bladder distention. Over time, this may lead to bile stasis and an irritant or stimulatory effect of the concentrated bile acids that may aid in the formation of sludge and mucus. Thyroid hormones also affect bile composition, which may contribute to gall bladder disease (Field and others 1986, Day and others 1989, Stravitz and others 1993). The effects of hypothyroidism on bile composition, such as decreased cholesterol and bile acid synthesis, can be overcome by environmental factors (Day and others 1989, Stravitz and others 1993). Hypothyroid rats fed a cholesterol-rich diet significantly increased biliary cholesterol secretion and reduced bile flow (Field and others 1986). This highlights the fact that one finding alone is unlikely to explain the disease process and may not be applicable in the face of other patient or environmental factors.

The diagnosis of hyperadrenocorticism has no gold-standard and has been a source of controversy (Behrend and others 2002). The definition of hyperadrenocorticism used in this study is similar to previous retrospective studies by Sepesy and others (2006) and Wood and others (2007). Subjectively, more dogs in the affected group had clinical or physical findings suggestive of undiagnosed hyperadrenocorticism but testing was not pursued or mortality prevented diagnostic efforts. It is possible that these factors resulted in underestimation of the endocrinopathy afflicted population. However, this is true in both the GBM group and the control group and theoretically, both had the same chance of diagnosis and missed diagnosis. The diagnosis of thyroid deficiency was variable in its testing, which is an inherent problem in retrospective studies. No dog was diagnosed based on a low thyroxine alone and all dogs not diagnosed historically were diagnosed by the internal medicine department. This increases our confidence in a true diagnosis of hypothyroidism, although a prospective evaluation with uniform testing would be ideal. With either hypothyroidism or hyperadrenocorticism, positive response to treatment would add strength to a diagnosis; however, this could not be consistently or reliably assessed in a retrospective study.

It is important to consider whether there could have been observation bias

inherent in the study with regards to the diagnosis of endocrinopathy. That is, did veterinarians look harder for endocrinopathies in the GBM dogs than in the control dogs once they knew that the dogs had GBM. We addressed this concern in several ways. First, we evaluated the proportion of dogs in each group that had testing for an endocrinopathy following the diagnosis of GBM/control disease (Table 3). It was determined that the proportion of dogs tested for hyperadrenocorticism after the diagnosis of GBM/control was not significantly different between the GBM and control groups. Thus, it does not appear that veterinarians were searching more rigorously for hyperadrenocorticism by testing more frequently in the dogs that had been diagnosed with GBM. The proportion of dogs tested for hypothyroidism after the diagnosis of GBM/control was significantly different between the GBM and control groups. So it is possible that veterinarians were making more attempts to diagnose hypothyroidism by testing more frequently in the dogs that had been diagnosed with GBM and thus the association between hypothyroidism and GBM is overestimated. However, it is also possible that the dogs in the GBM group had changes on history/physical examination/screening blood work that triggered screening by the veterinarian more frequently than dogs in the control group. We feel that the latter is more likely because the literature overtly suggesting a strong association between GBM and hypothyroidism is relatively recent compared with overall time frame of this study. The widely read literature suggesting endocrinopathy association with GBM did not occur until 2007 and 2008 representing a smaller portion of the study period and thus influence on veterinarians testing practices. The second method used to evaluate the possibility of observation bias was to determine how many visits each animal had to the veterinarian in the 6 months following the GBM/control diagnosis. If veterinarians were scrutinising the health status of dogs in the GBM group more carefully than dogs in the control group, one might expect GBM dogs to have more visits to the veterinarian in the 6 months following diagnosis. This was not the case; there was no significant

difference in the mean number of visits of GBM (2.4 visits) versus control dogs (2.6 visits) in the 6 months after GBM/control diagnosis. Our interpretation of these findings is that observation bias is highly unlikely in the hyperadrenocorticism dogs and possible, but not highly likely, in the hypothyroidism dogs.

There are several other limitations to this study. Gall bladder changes assessed by the ultrasonographer to be early or developing mucoceles, without further surgery or necropsy support, were excluded to avoid any overlap between gall bladder sludge and GBM. Biliary sludge is common in the canine patient population and there is no difference in its presence between healthy and sick dogs or dogs with other hepatobiliary diseases (Brömel and others 1998). The role of biliary sludge in the pathogenesis of GBM is unknown. The exclusion of these cases may have resulted in underestimation of GBM diagnosed exclusively by ultrasound. Due to the very specific control criteria and limitation of hospital cases available, exact breed matches were not always available and similar breed substitutions were made. This effect on the data is estimated to be minimal as substitution was necessary infrequently and other criteria were observed. An additional limitation pertains to the definition of an endocrinopathy which included a diagnosis made up to 6 months after surgery or ultrasound for both control and GBM dogs. This period was given to allow adequate time for potential work-up of endocrine disease in cases where it was suspected at or around the time of surgery or ultrasound but was delayed due to illness and recovery. It is possible that endocrine disease discovered within 6 months of presentation with GBM or other disease was newly acquired as opposed to being present during formation of GBM or other disease. Another limitation involves the inability to determine from a retrospective analysis, the level of disease control present in animals historically diagnosed and treated with endocrinopathies. This variable needs to be evaluated in a prospective manner to aid in determining whether control of these diseases reduces the odds of mucocele development or progression. A final limitation is that there were very low numbers of diabetes

mellitus cases included in this study and, therefore, the lack of statistical difference between the control and the GBM group could represent a type II error rather than a true lack of association.

We conclude that the odds of GBM in dogs with hypothyroidism and hyperadrenocorticism are significantly higher than in dogs without those endocrinopathies. These findings are very robust for the association between GBM and hyperadrenocorticism, based on the magnitude of the point estimate for hyperadrenocorticism, how far the lower end of the 95 per cent CI is removed from 0 and the fact that there is no evidence of observation bias. The findings for the association between GBM and hypothyroidism are less robust and further studies are needed to investigate the effects of steroid excess and thyroid insufficiency on canine bile *in vivo*. A prospective case-control or cohort study with testing of all animals would clearly further elucidate the relationship between GBM and endocrinopathies. We recommend that dogs diagnosed with GBM be screened for concurrent endocrinopathies if clinical suspicion is present. A heightened degree of suspicion of GBM should be present in dogs with pre-existing hyperadrenocorticism or hypothyroidism, which are presented for acute illness and typical biochemical changes.

## References

- AGUIRRE, A. L., CENTER, S. A., RANDOLPH, J. F., YEAGER, A. E., KEEGAN, A. M., HARVEY, H. J. & ERB, H. N. (2007) Gallbladder disease in Shetland Sheepdogs: 38 cases (1995-2005). *Journal of the American Veterinary Medical Association* **231**, 79-88
- AMSELLEM, P. M., SEIM, H. B. III, MACPHAIL, C. M., BRIGHT, R. M., TWEED, D. C., WRIGLEY, R. H. & MONNET, E. (2006) Long-term survival and risk factors associated with biliary surgery in dogs: 34 cases (1994-2004). *Journal of the American Veterinary Medical Association* **229**, 1451-1457
- BEHREND, E. N., KEMPPAINEN, R. J., CLARK, T. P., SALMAN, M. D. & PETERSON, M. E. (2002) Diagnosis of hyperadrenocorticism in dogs: a survey of internists and dermatologists. *Journal of the American Veterinary Medical Association* **220**, 1643-1649
- BENITAH, N., FELDMAN, E. C., KASS, P. H. & NELSON, R. W. (2005) Evaluation of serum 17-hydroxyprogesterone concentration after administration of ACTH in dogs with hyperadrenocorticism. *Journal of the American Veterinary Medical Association* **227**, 1095-1101
- BESSO, J. G., WRIGLEY, R. H., GLIATTO, J. M. & WEBSTER, C. R. (2000) Ultrasonographic appearance and clinical findings in 14 dogs with gallbladder mucocele. *Veterinary Radiology & Ultrasound* **41**, 261-271
- BREITENSTEIN, S., KRAUS, A. & CLAVIEN, P. (2006) Acute and chronic cholecystitis. In: *Diseases of the Gallbladder and Bile Ducts*. 2nd edn. Eds P Clavien and J. Baillie. Blackwell Publishing, Malden, MA, USA. pp 229-238

- BRÖMEL, C., BARTHEZ, P.Y., LÉVEILLÉ, R. & SCRIVANI, P.V. (1998) Prevalence of gallbladder sludge in dogs as assessed by ultrasonography. *Veterinary Radiology & Ultrasound* **39**, 206–210
- DAY, R., GEBHARD, R. L., SCHWARTZ, H. L., STRAIT, K. A., DUANE, W. C., STONE, B. G. & OPPENHEIMER, J. H. (1989) Time course of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and messenger ribonucleic acid, biliary lipid secretion, and hepatic cholesterol content in methimazole-treated hypothyroid and hypophysectomized rats after triiodothyronine administration: possible linkage of cholesterol synthesis to biliary secretion. *Endocrinology* **125**, 459–468
- FIELD, F. J., ALBRIGHT, E., MATHUR, S. N. (1986) The effect of hypothyroidism and thyroxine replacement on hepatic and intestinal HMG-CoA reductase and ACAT activities and biliary lipids in the rat. *Metabolism* **35**, 1085–1089
- GERMAN, S. V., LEMESHKO, Z. A., VAINSHEIN, T. I. & PETROVA, E. A. (1989) Bile excretion system in endogenous hypercorticism syndrome. *Sovetskaia Meditsina* **3**, 5–9
- HOLT, D. E., MEHLER, S., MAYHEW, P. D. & HENDRICK, M. J. (2004) Canine gallbladder infarction: 12 cases (1993–2003). *Veterinary Pathology* **41**, 416–418
- KLINE, L. W. & KARPINSKI, E. (2005) Progesterone inhibits gallbladder motility through multiple signaling pathways. *Steroids* **70**, 673–679
- KLINE, L. W. & KARPINSKI, E. (2008) Testosterone and dihydrotestosterone inhibit gallbladder motility through multiple signaling pathways. *Steroids* **73**, 1174–1180
- KLINKSPOOR, J. H., KUVER, R., SAVARD, C. E., ODA, D., AZZOUZ, H., TYTGAT, G. N., GROEN, A. K. & LEE, S. P. (1995) Model bile and bile salts accelerate mucin secretion by cultured dog gallbladder epithelial cells. *Gastroenterology* **109**, 264–274
- KUVER, R., SAVARD, C., ODA, D. & LEE, S. P. (1994) PGE generates intracellular cAMP and accelerates mucin secretion by cultured dog gallbladder epithelial cells. *The American Journal of Physiology* **267**, G998–G1003
- LAUKKARINEN, J., KOÖBI, P., KALLIOVALKAMA, J., SAND, J., MATILA, J., TURJANMAA, V., PÖRSTI, I. & NORDBACK, I. (2002a) Bile flow to the duodenum is reduced in hypothyreosis and enhanced in hyperthyreosis. *Neurogastroenterology and Motility* **14**, 183–188
- LAUKKARINEN, J., SAND, J., AITTOMÄKI, S., PÖRSTI, I., KOÖBI, P., KALLIOVALKAMA, J., SILVENNOINEN, O. & NORDBACK, I. (2002b) Mechanism of the prorelaxing effect of thyroxine on the sphincter of Oddi. *Scandinavian Journal of Gastroenterology* **37**, 667–73
- LAUKKARINEN, J., SAND, J., SAARISTO, R., SALMI, J., TURJANMAA, V., VEHKALAHTI, P. & NORDBACK, I. (2003) Is bile flow reduced in patients with hypothyroidism? *Surgery* **133**, 288–293
- MEHLER, S. J. & BENNETT, R. A. (2006) Canine extrahepatic biliary tract disease and surgery. *Compendium on Continuing Education for the Practicing Veterinarian* **4**, 302–314
- NEWELL, S. M., SELGER, B. A., MAHAFFEY, M. B., GRAY, M. L., JAMESON, P. H., CORNELIUS, L. M. & DOWNS, M. O. (1995) Gallbladder mucocele causing biliary obstruction in two dogs: ultrasonographic, scintigraphic, and pathological findings. *Journal of the American Animal Hospital Association* **31**, 467–472
- NORMAN, E. J., THOMPSON, H. & MOONEY, C. T. (1999) Dynamic adrenal function testing in eight dogs with hyperadrenocorticism associated with adrenocortical neoplasia. *Veterinary Record* **144**, 551–554
- PIKE, F. S., BERG, J., KING, N. W., PENNINGCK, D. G. & WEBSTER, C. R. (2004a) Gallbladder mucocele in dogs: 30 cases (2000–2002). *Journal of the American Veterinary Medical Association* **224**, 1615–1622
- PIKE, F. S., BERG, J., KING, N. W., PENNINGCK, D. G. & WEBSTER, C. R. (2004b) Revision to gallbladder mucocele article. *Journal of the American Veterinary Medical Association* **224**, 1916–1917
- POTAPOV, A. A., POTAPOVA, Z. G. & MEDVEDEV, M. A. (1976) Effect of adrenocortical insufficiency on the motor activity of the gall bladder. *Fiziologicheskii zhurnal SSSR imeni I. M. Sechenova* **62**, 1691–1697
- REGE, R. V. & PRYSTOWSKY, J. B. (1996) Inflammatory properties of bile from dogs with pigment gallstones. *American Journal of Surgery* **171**, 197–201
- REUSCH, C. E. (2005) Hyperadrenocorticism. In: *Textbook of Veterinary Internal Medicine*. 6th edn. Eds S. J. Ettinger and E. C. Feldman. Elsevier Saunders, St. Louis, MO, USA. pp 1592–1612
- SEPESEY, L. M., CENTER, S. A., RANDOLPH, J. F., WARNER, K. L. & ERB, H. N. (2006) Vacuolar hepatopathy in dogs: 336 cases (1993–2005). *Journal of the American Veterinary Medical Association* **229**, 246–252
- SHERLOCK, S. (2002) Gallstones and Inflammatory Gallbladder diseases. In: *Diseases of the Liver and Biliary System*. 11th edn. Eds S. Sherlock and J. Dooley. Blackwell Publishing, Oxford. pp 597–628
- STRAVITZ, R. T., HYLEMON, P. B., HEUMAN, D. M., HAGEY, L. R., SCHEINGART, C. D., TON-NU, H. T., HOFMANN, A. F. & VLAHCEVIC, Z. R. (1993) Transcriptional regulation of cholesterol 7 alpha-hydroxylase mRNA by conjugated bile acids in primary cultures of rat hepatocytes. *Journal of Biological Chemistry* **268**, 13987–13993
- WALTER, R., DUNN, M. E., d'ANJOU, M. A., & LECUYER, M. (2008) Nonsurgical resolution of gallbladder mucocele in two dogs. *Journal of the American Veterinary Medical Association* **232**, 1688–1693
- WOOD, F. D., POLLARD, R. E., UERLING, M. R. & FELDMAN, E. C. (2007) Diagnostic imaging findings and endocrine test results in dogs with pituitary-dependent hyperadrenocorticism that did or did not have neurologic abnormalities: 157 cases (1989–2005). *Journal of the American Veterinary Medical Association* **231**, 1081–1085
- WORLEY, D. R., HOTTINGER, H. A. & LAWRENCE, H. J. (2004) Surgical management of gallbladder mucoceles in dogs: 22 cases (1999–2003). *Journal of the American Veterinary Medical Association* **225**, 1418–1422
- YARIMAGAN, H. S. & BOR, N. M. (1986) Changes of bile lipid composition induced by methylprednisolone. *Biochemical Medicine and Metabolic Biology* **35**, 7–11
- ZEN, Y., HARADA, K., SASAKI, M., TSUNEYAMA, K., KATAYANAGI, K., YAMAMOTO, Y. & NAKANUMA, Y. (2002) Lipopolysaccharide induces overexpression of MUC2 and MUC5AC in cultured biliary epithelial cells: possible key phenomenon of hepatolithiasis. *The American Journal of Pathology* **161**, 1475–1484