

GALLBLADDER MUCOCELES IN DOGS:
retrospective evaluation of clinicopathological parameters
and different treatment modalities

23 CASES (2005-2013)

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Abstract

Objectives: To describe clinical features and long-term outcome and to determine risk and prognostic indicators in dogs diagnosed with gallbladder mucocele (GM).

Design: Retrospective study.

Animals: Twenty-three client-owned dogs of the referral patient population of the Utrecht University clinic in the period 2005-2013 in which a GM was diagnosed.

Methods: Data from medical records and follow-up were analysed, including signalment, medical history, physical examination findings, clinico- and histopathological findings, survival time, and prognostic factors for survival time.

Results: Dogs had a mean age of 8 years and gender was equally distributed. Eighteen dogs had a history of non-specific clinical signs (vomiting, anorexia, lethargy, polyuria and polydipsia, weight loss and diarrhoea) of acute or subacute onset. Physical examination findings revealed abnormalities in twenty-two dogs and included abdominal discomfort, icterus, abdominal distension, tachycardia, signs of dehydration and fever. Blood examination revealed a leucocytosis, an abnormally high alkaline phosphatase, alanine aminotransferase and fasting bile acids, and a hypoalbuminaemia in the majority of dogs. Thirteen dogs underwent cholecystectomy, two dogs were treated medically, and eight dogs received no treatment. Mortality due to the GM was 43.5% and estimated median survival time for the total group was 33 months. There was no significant difference in survival time between treatment groups. Risk factors for reduced survival were not identified.

Conclusion and clinical relevance: There is no difference in survival between dogs that underwent cholecystectomy and dogs that received no or medical treatment. Spontaneous resolution of clinical signs and the GM was reported. Rupture of the gallbladder warrants surgical intervention but does not preclude a positive outcome. A more expectative approach is in place for dogs without gallbladder rupture, since surgery inevitably leads to higher costs and more stress for the dogs and owners.

Introduction

A gallbladder mucocele (GM) is defined as the accumulation of a green-black, bile-laden, semisolid to immobile mucoid mass within the fundus of the gallbladder¹. Detection of a GM can be made during ultrasonographical evaluation (sometimes serendipitously), by histological examination following surgical removal or during post-mortem evaluation. Ultrasonographically, mucoceles are characterised by the appearance of a stellate or finely striated bile pattern and differ from biliary sludge by the absence of gravity dependent bile movement². This pattern, often referred to as a “kiwi fruit” gallbladder, appears to be pathognomonic for GM. Histologically, GM is characterised by hyperplasia of mucus-secreting glands within the gallbladder mucosa and abnormal accumulation of mucus within the gallbladder lumen¹.

Although seen rarely prior to the early 1990s, GM is now an increasingly recognised disease of the extrahepatic biliary tract in dogs^{1,3}. This increase in incidence might be in part the reflection of the incorporation of ultrasonography as a routine diagnostic modality². GMs are also reported, albeit rarely, in ferrets⁸ and humans⁹.

The pathogenesis of GM in dogs is unknown. It is unclear whether or not the cystic hyperplasia is a primary cause of GM formation or secondary to an underlying gallbladder dysfunction or dysmotility². Previous research data suggesting an inflammatory or bacterial aetiology have not been consistently supported by more recent findings¹. It has been suggested that biliary stasis or an increased concentration of bile salts in the gallbladder lumen encourage the formation of GMs^{1,7,10}. An association has been found between dyslipidemias and mucocele formation²; which includes idiopathic hyperlipidemia of certain breeds (Shetland Sheepdog and Miniature Schnauzer), as well as endocrinopathies known to influence lipid metabolism¹¹.

Published case reviews of GM in dogs have reported breed predilections for Cocker Spaniels, Shetland Sheepdogs and Miniature Schnauzers, possibly related to idiopathic hyperlipidemia in the latter two². GMs are more common in older dogs^{1,2,3,7}. No sex predilection has been reported so far.

Clinical features are often aspecific and patient presentation can further vary with other concurrent diseases and complications of the GM itself². The most common clinical signs are vomiting, anorexia, lethargy, polyuria and polydipsia, diarrhoea and abdominal distension^{1,3,7}.

On physical examination, more specific findings include abdominal pain, icterus, fever, tachypnea, and tachycardia^{1,3,7}. There is a wide variability in complete blood count (CBC) in these dogs and it is usually unremarkable¹². Serum biochemistry panels may reveal elevated activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and gammaglutamyl transpeptidase (GGT), as well as increased concentrations of blood urea nitrogen (BUN), total bilirubin and bile acids^{1,6,12}.

Most authors recommend cholecystectomy as the preferred treatment for GM¹⁻⁶. This recommendation is based on histological findings, which suggests that the gallbladder itself is diseased, the fact that the mucocele content is unlikely to pass from the gallbladder under the influence of cholergics, the fact that mucoceles may lead to pressure necrosis and subsequent rupture of the gallbladder wall and the fact that GMs can be complicated by potentially fatal secondary bacterial infections¹⁻⁵. For uncomplicated GM a laparoscopic technique is a possibility⁴. Survival following surgery tends to be guarded. The perioperative mortality rate ranges from 21% to 32%⁶. The highest level of risk is associated with the early post-operative period, and dogs that survive this period are considered to have a good prognosis¹³. Commonly encountered causes of perioperative death includes sepsis, disseminated intravascular coagulation, surgical site dehiscence and pancreatitis^{7,10}. Significant risk factors includes increased ALP and GGT activities and increased bilirubin concentrations^{10,13}. Less is known about medical management of GMs. Medical treatment is generally reserved for animals lacking clinical signs or for those whose owners are unable or unwilling to pursue surgery². Resolution of GM is reported in dogs with medical treatment only⁶. In other cases, the GM can remain static for a long period of time or may transform into an acute clinical condition².

Few reports of GM exist in the veterinary medical literature, and these are restricted to data of patients in the United States and Canada^{1-7,10-13}. The purpose of this study is two-fold. First, to describe signalment, medical history, ultrasonographical, clinical- and histopathological findings of dogs diagnosed with GM in the Dutch referral population. Secondly, the study is designed to assess outcome and survival after diagnosis and treatment and to identify prognostic factors observed at initial presentation for reduced survival times.

Materials and methods

Patients

Patients in this study were referred between December 2005 and July 2013 to the Department of Clinical Sciences of Companion Animals (DCSCA), Utrecht University, the Netherlands. Dogs that had ultrasonographical findings characteristic of GM, and/or dogs that underwent cholecystectomy or post-mortem examination and had a diagnosis of GM confirmed histologically, were reviewed prior to inclusion in the study. Reports on ultrasonography and histopathological examinations were completed by a board certified veterinary radiologist and pathologist, respectively.

Data collection

Medical records of selected patients were obtained and reviewed using a computer database (Vetware®). Information included signalment, medical history, findings of physical examination, laboratory findings, ultrasonographical findings, surgical intervention and medical treatment, intra-operative findings, results of histopathological examination and clinical outcome. Long-term follow-up information concerning surviving dogs was obtained via telephone interviews with owners and/or referring veterinarians. Follow-up information included clinical state after the final consultation at the DCSCA, survival time after diagnosis and presumed cause of death.

Statistical analyses

Analyses were performed using a commercially available software package (SPSS Benelux BV, Gorinchem, the Netherlands) and with standard statistical methods^{14,15,16}. Scatter and box-and-whisker plots were used to evaluate data distribution and to identify outliers. The Chi Square-test was performed for goodness-of-fit of the study group compared with the total canine clinical population (2005–20013) for breed and sex distribution. Survival fractions were calculated according to the Kaplan-Meier estimate procedure. The survival period was defined as the interval between the date of initial diagnosis and the date of death due to any cause. Dogs that had died of GM-related causes were counted as events. Dogs that had died from non-GM related causes and dogs that were still alive at the time of follow-up were counted as censored cases. Survival data are depicted as the estimated median survival time (EMST) \pm SE (95% confidence interval). Differences between Kaplan-Meier curves for the three different treatment groups (surgically treated, treated with ursodeoxycholic acid or no

treatment) were tested for significance ($P < 0.05$) using a Mantel-Cox log rank test. These groups were those without medical treatment, treatment with ursodeoxycholic acid only and surgical intervention. Prognostic factors for reduced survival time were analysed by a univariate Cox's proportional-hazard analysis. The following (groups of) variables were analysed: age, presence and duration of clinical signs, clinico-chemical and haematological parameters, findings at ultrasonography and surgery and histopathologic examination findings. All variables that were significant or approached significance ($P < 0.10$) were entered into a stepwise multivariate Cox's proportional-hazard analysis with backwards removal of variables (likelihood ratio). Secondly, all variables that had a hazard ratio above three were entered into a stepwise multivariate Cox's proportional-hazard analysis with backwards removal of variables (likelihood ratio). Various multivariate analyses were executed, since not all analysed variables were available for all patients included in the study. Variables with lower numbers of cases and events were entered separately for maximal output, and to prevent an unnecessary reduction of cases. Data are presented as P-values, hazard ratios (HR) and 95% confidence intervals (95%CI).

Results

Signalment

Twenty-three dogs met the criteria for inclusion in the study. Ten (43.5%) were sexually intact males, four (17.4%) were castrated males, one (4.3%) was a sexually intact female and eight (34.8%) were spayed females. The distribution of genders in the study population did not significantly differ from the clinic population ($P=0.623$). Mean \pm SD (range) age of dogs was 9.2 ± 3.3 (2.3-16.8) years. Mean \pm SD body weight was 15.8 kg (34.8 lb) \pm 11 kg (24.2 lb). Of the 23 dogs included in the study, 4 (17.4%) were Beagles (of which two were siblings), 2 (8.7%) each were Bernese Mountain dogs, Miniature Schnauzer and mixed-breeds and 1 (4.3%) each were Border Terrier, Jack Russel Terrier, Scottish Terrier, Dachshund, Cavalier King Charles Spaniel, English Cocker Spaniel, Keeshond, Nova Scotia Duck Tolling Retriever, Polish Lowland Sheepdog, Dutch Shepherd Dog, White Shepherd Dog, Prague Ratter and Chihuahua. The breed distribution of the study population did not significantly differ from the clinic population ($P=0.162$).

Medical history

Fourteen patients had a history of concurrent disease and were treated accordingly; heart diseases ($n=3$), eye disorders ($n=3$), idiopathic immune-mediated haemolytic anaemia ($n=2$), hypothyroidism ($n=2$), hyperadrenocorticism ($n=2$), diabetes mellitus ($n=2$), epilepsy ($n=1$), metastasised liver cell carcinoma ($n=1$) and Leydigcell tumour ($n=1$). In one dog GM was found serendipitously and four dogs were referred for medical work-up of abnormalities found in blood examinations indicative of hepatic disease, although these dogs did not have a history of clinical signs. Eighteen dogs were referred with clinical signs, mostly of acute or subacute onset. The mean \pm SD (range) duration from onset of clinical signs to diagnosis of GM was 8.1 ± 9.1 (0 to 30) days. Clinical signs reported in the history were mainly non-specific, including lethargy (69.6%), inappetance or anorexia (65.2%), vomiting (60.9%), polyuria and polydipsia (34.8%), weight loss (34.8%) and diarrhoea (30.4%). Fewer patients presented with melena (21.7%) and/or a distended abdomen (13%).

Physical examination

Four dogs were obese (18.2%) and three dogs presented with sopor (13.6%). No abnormalities were detected during physical examination in one dog. Common findings upon physical examination in the other dogs were discomfort during palpation of the abdomen

(68.2%), icterus (31.8%), a distended abdomen (27.3%), tachycardia (27.3%), signs of dehydration (22.7%), fever (22.7%) and hepatomegaly (22.7%). Less commonly encountered were tachypnea (18.2%) and/or positive abdominal undulation (9.1%).

Blood examination

Complete CBCs were not performed in all dogs and analyses were selected for each dog separately. Values that were measured upon initial diagnosis are summarised in Table 1.

Table 1: haematology values at initial diagnosis.

Parameter (reference interval)	Mean \pm SD (range)	No.	Elevated	Decreased
Haematocrit (0.42-0.61 L/L)	0.42 \pm 0.06 (0.25 - 0.50)	16	0/16	7/16
MCV (63.5-72.9 fL)	66.0 \pm 1.9 (64.0 - 69.6)	7	0/7	0/7
MCHC (20.5-22.4 mmol/L)	21.8 \pm 0.7 (21.0 - 22.6)	7	2/7	0/7
MCH (1.37-1.57 fmol/L)	1.4 \pm 0.1 (1.4 - 1.5)	7	1/7	0/7
Leukocytes (4.5-14.6 x10 ⁹ /L)	19.4 \pm 11.7 (8.6 - 46.3)	14	9/14	0/14
Segments (2.9-11.0 x10 ⁹ /L)	15.4 \pm 11.7 (3.5 - 43.0)	14	8/14	0/14
Rods (0.0-0.3 x10 ⁹ /L)	0.3 \pm 0.6 (0.0 - 1.9)	14	2/14	NA
Juveniles (0.0 x10 ⁹ /L)	0.0 \pm 0.0 (0.0 - 0.0)	14	0/14	NA
Lymphocytes (0.8-4.7 x10 ⁹ /L)	2.0 \pm 1.2 (0.0 - 3.7)	14	0/14	2/14
Monocytes (0.0-0.9 x10 ⁹ /L)	1.4 \pm 0.8 (0.5 - 3.2)	14	10/14	NA
Eosinophils (0.0-1.6 x10 ⁹ /L)	0.2 \pm 0.4 (0 - 1.5)	14	0/14	NA
Basophils (0.0-0.1 x10 ⁹ /L)	0.0 \pm 0.0 (0.0 - 0.1)	14	0/14	NA
Thrombocytes (144-603 x 10 ⁹ /L)	353.2 \pm 235.3 (100-771)	12	2/12	1/12
APTT (10-17.2 s)	18.2 \pm 8.4 (9.4 - 43.1)	20	7/20	1/20
PT (6.7-9.5 s)	7.7 \pm 2.1 (5.8 - 14)	20	2/20	7/20
Fibrinogen (1-2.8 g/L)	4.2 \pm 2.2 (0.6 - 8.1)	19	14/19	2/19

SD = standard deviation; No. = number of blood values available; low = proportion of values less than reference limit; high = proportion of values greater than reference limit; NA = not applicable. MCV = mean corpuscular volume.

Seven dogs (44%) were anaemic, nine had a leucocytosis (64.3%), eight had neutrophilia (57.1%) and ten had a monocytosis (71.4%). Less frequently encountered abnormalities were

high mean corpuscular haemoglobin concentrations (MCHC) in two dogs (28.6%), high mean corpuscular haemoglobin (MCH) in one dog (14.3%), high rod count in two dogs (14.3%) and lymphopenia in two dogs (14.3%). Two dogs had a high thrombocyte count (16.7%) whereas one dog had a low thrombocyte count (8.3%).

Clinical chemical tests were not performed in all dogs and analyses were selected for each dog separately. Values that were measured upon initial diagnosis are summarised in Table 2.

Table 2: clinical chemistry values at initial diagnosis.

Parameter (reference interval)	Mean \pm SD (range)	No.	Elevated	Decreased
Urea (3.0-12.5 mmol/L)	10.5 \pm 16.5 (2.2 – 64.4)	13	2/13	2/13
Creatinin (50-129 μ mol/L)	108.5 \pm 137.1 (37.0 – 560.0)	13	1/13	2/13
Glucose (4.5 -5.8 mmol/L)	16.4 \pm 24.1 (5.3 – 70.3)	7	4/7	0/7
ALP (0-89 U/L)	2659.3 \pm 3100.5 (54 - 9230)	16	14/16	NA
ALP-65 (0-73 U/L)	1055.2 \pm 1404.6 (6 - 4180)	14	12/14	NA
ALT (0-70U/L)	414.9 \pm 918.6 (15 - 3315)	12	9/12	NA
Bile Acids (fast) (0-10 μ mol/L)	169.3 \pm 278.5 (1 - 1030)	17	10/17	NA
Total protein (55-72 g/L)	60.5 \pm 11.7 (36 - 74)	15	2/15	4/15
Albumin (26-37 g/L)	23.3 \pm 7.2 (11 - 33)	15	0/15	9/15
Sodium (141-150 mmol/L)	142.5 \pm 6.1 (129-151)	14	1/14	5/14
Potassium (3.6-5.6 mmol/L)	4.0 \pm 1.0 (2.6 – 6.4)	14	1/14	5/14
Calcium (1.98-2.97 mmol/L)	2.3 \pm 0.6 (1.2 – 2.9)	9	0/9	2/9
Phosphate (0.65-2.12 mmol/L)	1.9 \pm 1.5 (1.2 – 5.3)	7	1/7	0/7

SD = standard deviation; No. = number of blood values available; low = proportion of values less than reference limit; high = proportion of values greater than reference limit; NA = not applicable; (fast) = fasting bile acids.

Most frequently encountered were an abnormal high alkaline phosphatase (ALP) in fourteen dogs (87.5%) and elevated alanine aminotransferase (ALT) levels in nine dogs (75%). Ten dogs had elevated fasting bile acids (58.8%) and nine dogs had a hypoalbuminemia (60%). Electrolyte levels were normal in the majority of dogs, although five dogs (35.7%) had a hyponatremia and hypokalemia. Two dogs (22.2%) had a hypocalcemia. Coagulation was considered to be retarded in seven dogs (35%) as measured by the activated partial

thromboplastin time (APTT) but only two dogs (10%) had a prolonged prothrombin time (PT). Fibrinogen concentrations were elevated in fourteen dogs (73.7%) but lowered in two dogs (10.5%). Mean glucose concentration of the group was considered abnormally high compared to the reference interval. One outlier was identified with a glucose level of 70.3 mmol/L. Excluding this outlier resulted in a mean \pm SD (range) glucose level of 7.5 ± 4.5 (5.3-16.7) mmol/L and three dogs with a hyperglycaemia (50%).

Urine analysis

In eight dogs the urinary samples were investigated. These revealed that the mean \pm SD (range) of the specific gravity was 1.019 ± 0.011 (1.010 – 1.040). Mean \pm SD (range) pH was 6.5 ± 0.7 (5.0 – 7.1). Four dogs (50%) had a proteinuria, four (50%) had a haemoglobinuria and one dog had a glucosuria (14.3%).

Ultrasonographic examination

Abdominal ultrasonography was performed in all dogs (n=23) during the initial diagnostic evaluation. One dog had ultrasonographical features resulting in a clinical diagnosis of cholecystitis and the post-mortem evaluation revealed the GM. Twenty-two dogs (95.7%) had ultrasonographic features consistent with a GM (central echogenic immobile gallbladder content with a striated, stellate or mixed pattern and an anechoic periphery). The gallbladder was subjectively considered distended in twelve (54.5%) dogs and five (22.7%) dogs had a thickened gallbladder wall. Focal peritonitis was found in nine (40.9%) dogs based on the presence of hyperechoic cranial mesenteric fat and/or fluid surrounding the gallbladder, but biliary tract rupture was suspected in only four of these nine dogs. The common bile duct could be visualised in two (9.1%) dogs, suggesting that it was distended. Abnormalities of the liver were present in nine dogs, of which six (27.3%) an increased hepatic size, seven (31.8%) an irregular parenchyma and one (4.5%) dog showed signs of intrahepatic cholestasis. Other abdominal organs were usually unremarkable but encountered abnormalities were pancreatitis (n=1, 4.5%) and splenomegaly (n=3, 13.6%), of which two with an irregular splenic parenchyma. During ultrasonography cholecystocentesis was performed for bacterial culture in five dogs, of which none yielded a positive result. In three dogs liver biopsy was performed under ultrasonographic guidance.

Therapy and clinical outcome

Of the twenty-three dogs, one dog was directly euthanized upon initial diagnosis due to the severity and deteriorating clinical status and because of the owner's preference not to pursue surgery. Six dogs received no treatment (expectative). Two dogs received medical treatment consisting of only ursodeoxycholic acid (15mg/kg, PO, divided into 2 doses/day). One dog was admitted to the intensive care because of severe uncontrollable hyperglycaemia, ketoacidosis and acute renal failure. This dog was euthanised three days later because of deteriorating clinical status despite medical therapy. Reasons for no or medical treatment were other medical conditions that needed stabilisation and treatment first, owner's refusal or inability to pursue surgery and the lack of clinical signs. Thirteen dogs were treated surgically. Five dogs underwent emergency explorative celiotomy because biliary tract rupture was suspected or thought to be imminent on the basis of ultrasonography. Eight dogs underwent surgery without emergency indication with a mean \pm SD (range) duration between initial diagnosis and surgery of 126.1 ± 165.9 (1-387) days. Of these eight dogs three dogs were treated medically with ursodeoxycholic acid (15 to 40 mg/kg, PO, divided into 2 doses/day) and one dog underwent a cholecystoduodenostomy, prior to cholecystectomy. During surgery macroscopic aspects of the abdomen were evaluated. Three out of four ultrasonographical suspected biliary tract ruptures were confirmed of which two with a gallbladder rupture. The third dog had an intact gallbladder but multiple lacerations and necrosis of the common and cystic bile ducts. This dog was euthanized during surgery. All three dogs had diffuse bile peritonitis, although it had a more chronic aspect in one dog. Focal peritonitis with adhesions of the gallbladder to the liver, omentum and/or falciform ligament was present in five of the remaining ten dogs. Macroscopic appearance of the gallbladder was abnormal in twelve dogs. Reported abnormalities were an enlargement (n=6), firm content (n=7), thickened wall (n=3) and preperforative aspects (n=2). Nine dogs had a distended common bile duct and seven dogs had a distended cystic bile duct. Hepatomegaly (n=2), irregular liver surface (n=2), splenomegaly (n=1), cysts in the jejunum (n=1), distended stomach (n=1) and a bile-filled thickness in the duodenum not connected to the common bile duct (n=1) were reported abnormalities of other abdominal organs. Bile was taken during surgery from three dogs for bacterial culture, of which one yielded a positive result (*Micrococcus*). Before cholecystectomy was performed, patency of the common bile duct was checked in most patients via cholecystotomy (n=6) or duodenotomy (n=3). In one dog manual manipulation was necessary to remove an obstruction (concrement) from the common bile

duct, after which patency was established. Cholecystectomy was then performed twelve dogs (one was euthanized before cholecystectomy). All dogs were admitted to the intensive care after surgery for recovery of anaesthesia, fluid therapy, pain medication and antibiotics if indicated. One dog that underwent emergency cholecystectomy did not recover from general anaesthesia and died five hours after surgery with hyperthermia and tachycardia. Post-mortem evaluation (only performed on liver and spleen specimen) could not reveal any cause of this sudden death. One dog returned to the clinic thirteen days after surgery because of severe anaemia (most likely immune-mediated) and wound dehiscence. This dog was given a blood transfusion and operated for a second time because of this complication. Antibiotics, pain medication, corticosteroids and fluid therapy (including a second blood transfusion) were administered during the post-operative intensive care period. Despite this, the dog died four days after the second surgery with signs of disseminated intravascular coagulation. Nonfatal post-operative complications were anaemia because of intra-operative blood loss (n=1), wound infection (n=1) and development of neurological signs (n=1).

Histopathological examination

Histological analysis of the gallbladder was available in twelve dogs following surgical excision (n=11) or necropsy (n=1). Eleven gallbladders had (cystic) hyperplasia of mucosal mucus-secreting glands and abnormal accumulation of mucus within the gallbladder lumen, confirming the diagnosis of GM. Cholecystitis was present in ten dogs of which eight in combination with gallbladder wall necrosis. Liver specimen for histologic evaluation were available in ten dogs, which was obtained via biopsy under ultrasonographical guidance (n=3), during surgery (n=6) or during necropsy (n=1). Typical histologic findings included fibrosis (n=5), variable forms of hepatitis (n=5), bile duct hyperplasia (n=4), cholangitis (n=4), necrosis (n=3) and hepatocellular vacuolar change (n=2). Two dogs had histopathological signs of pancreatitis, of which one also had ultrasonographic signs of pancreatitis.

Survival

Four dogs died within three days of initial diagnosis of which three (two that underwent emergency cholecystectomy) were related to the GM. The fourth dog was euthanized because of severe uncontrollable hyperglycaemia, ketoacidosis and acute renal failure. One dog was euthanized 38 days after initial diagnosis because of surgical complications. All eighteen surviving dogs could be followed up via owners and/or referring veterinarians. Six dogs were

still alive at follow up (of which two that underwent emergency cholecystectomy), six dogs died or were euthanized because of GM related clinical signs and six dogs died of other causes (of which one that underwent emergency cholecystectomy). Among these were uncontrollable epileptic attacks (n=1), metastasized liver cell carcinoma (n=1), renal failure (n=1), adrenal adenocarcinoma (n=1) and sudden death of unknown cause (n=2). The estimated median survival time (EMST) of the total group was 33.0 ± 13.0 months (95%CI, 7.6-58.4), with ten dogs counted as events (43.5%).

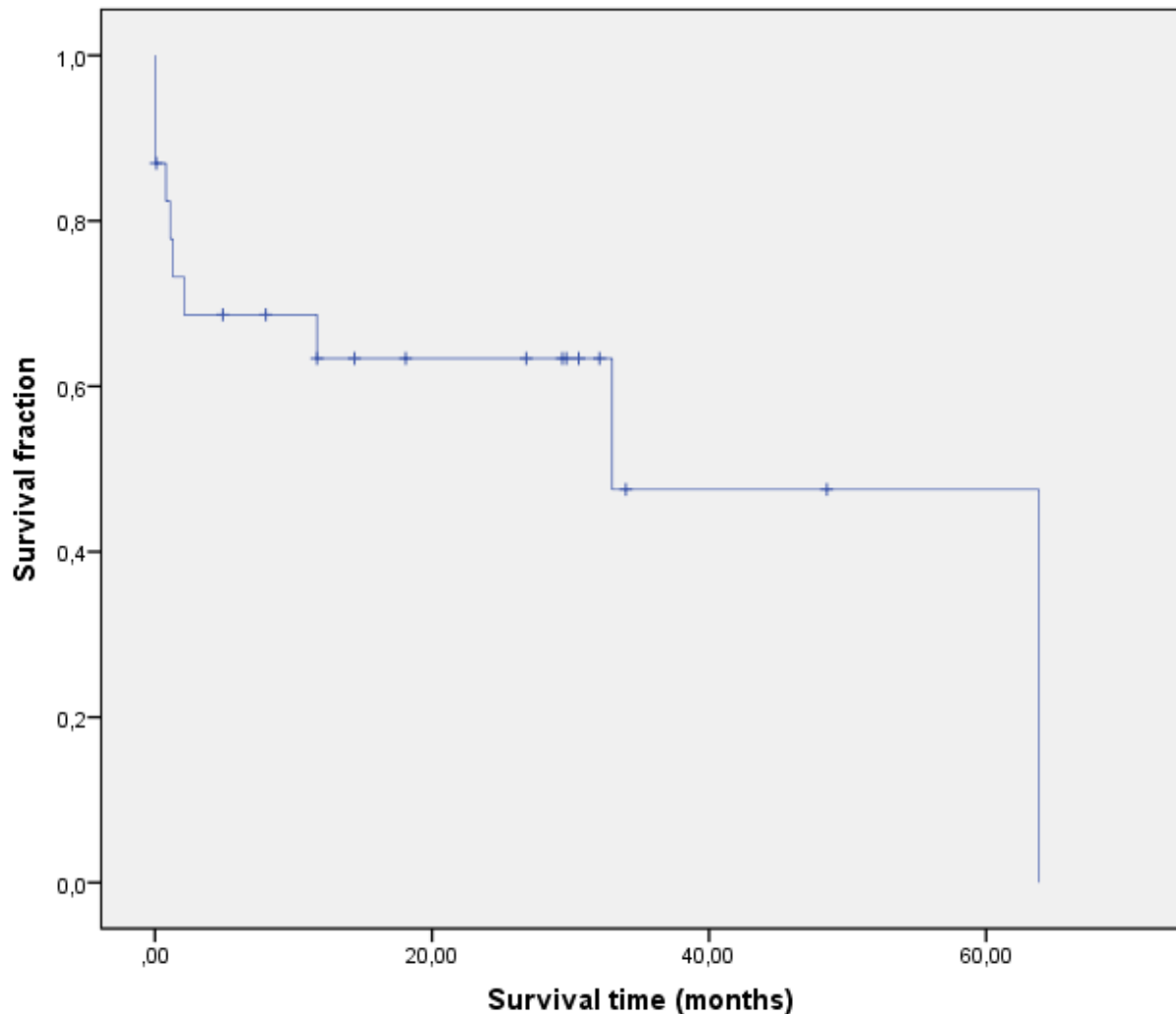


Figure 1: Survival curve calculated with the Kaplan-Meier estimate procedure for survival time in months after initial diagnosis of GM for the total group (n=23, 2005-2013). Censored cases (i.e. dogs that died from unrelated causes or were still alive at the time of follow-up) are represented by vertical bars.

EMST's were compared between different treatment groups (no treatment, treatment with ursodeoxycholic acid or treated by surgical intervention). In the ursodeoxycholic acid treatment group both cases were censored so no statistics could be given. In the group of dogs that received no treatment (n=8), four were counted as events (50%). This group had an

EMST of 2.1 ± 1.3 months (95%CI, 0.0-4.6). Of the thirteen dogs that received surgical treatment, six dogs were counted as events (46.2%). The EMST of this group was 63.8 ± 0.0 (95%CI could not be given). Despite this difference, comparison of the EMST's between the latter two groups using a Mantel-Cox log rank test did not yield a significant difference ($P=0.253$). Another comparison was made between patients that underwent emergency cholecystectomy ($n=5$) and patients that underwent cholecystectomy without an emergency indication ($n=8$). In the group without an emergency cholecystectomy four patients (50.0%) were counted as events. The EMST of this group was 63.8 ± 0.0 (95%CI could not be given). In the emergency cholecystectomy group two patients were counted as events (40.0%) but an EMST could not be given due to the low number of events. A Mantel-Cox log rank test did therefor also not yield a significant difference ($P=0.499$).

Prognostic factors

Univariate Cox's proportional-hazard analyses were performed to screen for variables with a prognostic value for reduced survival (Table 3).

Table 3: Variables in the univariate Cox's proportional hazard analysis for survival time after initial diagnosis ($n=23$, 2005-2013).

Variable	No.	Events	P	HR	95%CI
<u>Signalment:</u>					
Age	23	10	0.972	0.97	0.82 - 1.23
<u>Clinical signs:</u>					
Duration	23	10	0.848	1.01	0.94 - 1.08
Lethargy	23	10	0.576	1.57	0.33 - 7.56
Vomiting	23	10	0.546	1.54	0.38 - 6.16
Anorexia/inappetance	23	10	0.880	1.13	0.28 - 4.48
Polyuria/polydipsia	23	10	0.399	0.51	0.10 - 2.46
Weight loss	23	10	0.408	1.80	0.45 - 7.23
Diarrhoea	23	10	0.357	1.97	0.46 - 8.40
Distended abdomen	23	10	0.081*	3.29*	0.86 - 12.55
Abdominal discomfort	23	10	0.355	2.11	0.43 - 10.22
Icterus	23	10	0.123	0.19	0.02 - 1.56
Tachycardia	23	10	0.765	1.24	0.30 - 5.06
Dehydration	23	10	0.097*	3.16*	0.81 - 12.27
Fever	23	10	0.768	1.27	0.26 - 6.33
Hepatomegaly	23	10	0.821	0.83	0.17 - 4.03
Tachypnea	23	10	0.687	0.65	0.08 - 5.29
Positive abdominal undulation	23	10	0.881	1.18	0.14 - 9.81

No. = number of dogs; Events = all cases with GM related death; HR = hazard ratio; CI = confidence interval; * variables with $P < 0.10$ and/or $HR > 3$.

Table 3 cont'd: Variables in the univariate Cox's proportional hazard analysis for survival time after initial diagnosis (n=23, 2005-2013).

Variable	No.	Events	P	HR	95%CI
<u>Blood examination</u>					
Anaemia	16	6	0.238	2.79	0.51 - 15.31
Leukocytosis	14	6	0.199	4.11*	0.48 - 35.61
Neutrophilia	14	6	0.199	4.11*	0.48 - 35.61
Monocytosis	14	6	0.876	0.87	0.16 - 4.78
Elevated ALP	16	8	0.887	0.86	0.01 - 7.36
Elevated ALT	12	6	0.645	0.67	0.12 - 3.67
Elevated bile acids (fast)	17	9	0.992	0.99	0.22 - 4.44
Hypoalbuminemia	15	8	0.210	3.88*	0.47 - 32.30
APTT (prolonged)	20	10	0.231	2.37	0.58 - 9.74
PT (prolonged)	20	10	0.752	1.40	0.17 - 11.47
Sodium (low)	14	7	0.173	3.21*	0.60 - 17.11
Potassium (low)	14	7	0.993	1.01	0.18 - 5.52
Calcium (low)	9	4	0.391	0.22	0.00 - 133.79
<u>Ultrasonographic examination</u>					
Distended gallbladder	23	10	0.486	0.54	0.09 - 3.12
Thickened wall	23	10	0.721	1.66	0.10 - 27.02
Focal peritonitis	23	10	0.946	1.07	0.14 - 8.23
Suspected rupture	23	10	0.964	1.06	0.10 - 11.56
Distended common bile duct	23	10	0.834	1.26	0.15 - 10.62
Hepatomegaly	23	10	0.942	1.07	0.17 - 6.57
Abnormal liver paranchym	23	10	0.725	1.31	0.29 - 5.91
Pancreatitis	23	10	0.273	3.51*	0.37 - 33.17
Peritoneal effusion	23	10	0.830	1.28	0.14 - 11.83
<u>Findings upon surgery</u>					
Biliary tract rupture	13	5	0.458	809.0*	0.00 - 3.94*10 ¹⁰
Focal peritonitis with adhesions	13	5	0.521	0.04	0.00 - 1.09*10 ⁵
Preperforative aspects	13	5	0.893	0.76	0.14 - 41.81
Distended gallbladder	13	5	0.225	0.18	0.10 - 2.92
Distended common bile duct	13	5	0.413	1267.0*	0.00 - 3.41*10 ¹⁰
Distended cystic bile duct	13	5	0.260	11.39*	0.17 - 786.81
<u>Histopathological findings</u>					
Gallbladder wall necrosis	12	5	0.326	0.00	0.00 - 658.72
Cholecystitis	12	5	0.783	0.00	0.00 - 1.96*10 ²⁶
Hepatitis	10	4	0.650	0.569	0.05 - 6.52
Hepatic fibrosis	10	4	0.933	0.00	0.00 - .

No. = number of dogs; Events = all cases with GM related death; HR = hazard ratio; CI = confidence interval; * variables with P < 0.10 and/or HR > 3.0.

The factors distended abdomen and signs of dehydration present at the time of initial diagnosis had a P-value < 0.10. The variables distended abdomen, signs of dehydration, pancreatitis, leucocytosis, neutrophilia, hypoalbuminaemia, hyponatremia, biliary tract rupture and distended common and cystic bile ducts had hazard ratios above three. Unfortunately, combining these variables in multivariate analyses generated no models with significant prognostic factors for reduced survival.

Discussion

Twenty-three dogs were diagnosed with GM in the period of December 2005- July 2013 following referral to the DCSCA, Utrecht University, the Netherlands. In this group of dogs, gender was equally distributed. Although long-term administration of a progestational drug has been reported to result in a GM in a dog²⁶, the lack of an apparent female predisposition for the condition, inclusion of only one sexually intact female and the absence of history of administration of these drugs in this study does not support this aetiopathogenesis. Dogs were usually middle-aged or older, consistent with previous reports^{1,3,13}. Possible breed predispositions for GM could not be statistically proven. As this was the case for most parameters in this study it might be the result of the low number of cases included in the study. Especially since findings do suggest a breed predisposition for Beagles as four of the twenty-three in this study were Beagles and only 0.02% of the total clinic population are Beagles. None of the Beagles in this study had concurrent diseases (such as endocrinopathies¹¹) that could account for a higher risk of GM in these dogs and other aetiologic factors could not be identified. ABCB4 gene mutations are associated with GM formation in Shetland Sheepdogs but have not been reported for Beagles¹⁷. In contrast, previously reported other breed predispositions for Cocker Spaniels, Miniature Schnauzers and Shetland Sheepdogs^{1,2,3} could not be confirmed with the study reported here. Existence and aetiology of breed predisposition for GM (especially in Beagles) might be an interesting subject for further research..

A subset of dogs with GM in the present study had additional endocrinopathies (ie, two dogs had hyperadrenocorticism, two dogs had hypothyroidism and two dogs had diabetes mellitus). Multiple previous studies have suggested a possible association between gallbladder disease and select endocrinopathies^{1,2,11,13,28,29}, and hyperadrenocorticism is the endocrinopathy with the highest prevalence in dogs with GM¹¹. Although an association has been suggested, no clear role of endocrinopathies in the pathogenesis of GM has been proposed in dogs. Immunosuppression, as associated with high levels of corticosteroids, resulting in cholecystitis and subsequent increase in mucus secretion has been proposed as an aetiopathogenic factor¹¹. Although bacterial infection or inflammation may play a contributing role in GM formation, it is unlikely that this is a key feature of pathogenesis as 20 to 55 per cent of GM show no signs of inflammation on histopathological examination^{2,13}. Bile or gall bladder samples from mucoceles only contained culturable bacteria in 9 to 35 per cent of cases in recent reports^{1,7,13}. These results are consistent with the present study. Most

dogs had histopathological signs of inflammation (10/12), but it was assumed that this was likely the result of necrosis, present in most dogs (8/10). Bacterial culture of the bile was positive in only one of eight dogs. It has also been reported that patients with diabetes mellitus and hypothyroidism have delayed gallbladder emptying, as the result of hypomotility of the gallbladder and decreased relaxation of the sphincter of Oddi. Prolonged exposure to bile salts may cause formation of a GM^{35,36}. Motility of the gallbladder has not been measured in this study. Finally, thyroid hormones and corticosteroids also affect bile composition¹³, which may contribute to gall bladder disease. However, the effects on bile composition can be overcome by environmental factors (e.g., diet)^{32,33}. This highlights the fact that one finding alone is unlikely to explain the disease process and may not be applicable to other patient or environmental factors.

Most dogs presented with clinical signs of acute onset (mean=8 days) and although non-specific, these findings are consistent with previous reports^{1,3,7} and included lethargy, inappetance/anorexia, vomiting, polyuria/polydipsia, weight loss and diarrhoea. None of these symptoms or the duration of clinical signs were prognostic factors for reduced survival. Upon physical examination two-third of the dogs showed discomfort during palpation of the abdomen. Historically, it had been assumed that dogs with biliary tract disorders have minimal abdominal discomfort⁷ although in humans it is associated with chronic pain in the right upper quadrant of the abdomen¹⁸. In more recent studies, it is recognized that signs of abdominal pain can be associated with disease of the biliary tract⁷, but concurrent pancreatitis as a cause of these could not be ruled out^{1,13}. In the current study, only one of the fifteen dogs with abdominal discomfort had ultrasonographical signs of a pancreatitis, so abdominal discomfort it is most likely associated with a GM. However, none of the dogs were tested for a pancreatitis and clinical signs are alike, so a certain association is difficult to prove. Other frequently encountered abnormalities upon physical examination were icterus, abdominal distension, tachycardia and fever, consistent with previous reports^{1,2,3}. None of the clinical signs reported in the history or found upon physical examination appeared to be of prognostic value for reduced survival, or were specific for gallbladder rupture. According to the owners, five dogs did not have clinical signs that could be related to a GM. These dogs were referred because of other diseases or elevated liver enzymes in blood examinations without clinical signs. However, upon physical examination in our clinic only one dog did not have any signs of a GM and diagnosis of the GM during abdominal ultrasonography was serendipitous. The other dogs had fever, icterus, abdominal distension and/or discomfort. Although a GM can be

subclinical, absence of clinical signs in the history might merely reflect an inability of owners (and referring veterinarians) to recognise these signs. No association has been found between owner's recognition of clinical signs and survival.

Results of CBC's are usually within reference range⁶, although anaemia, neutrophilic leucocytosis and monocytosis have been reported^{1,3}. High white blood cell (WBC) counts are associated with gallbladder rupture and bile peritonitis³. The present study revealed CBC abnormalities in more dogs than expected (71.4%), but abnormalities itself were consistent with previous studies. Anaemia was present in approximately half of the dogs. WBC's revealed a leucocytosis in most dogs, with a monocytosis and neutrophilia as most frequently encountered. Leucocytosis with a left-shift was present in two dogs, both of which had a gallbladder rupture. For the third dog with a gallbladder rupture blood examination data were not available. Although based on minimal data, findings of a leucocytosis with a left-shift should raise suspicion of a gallbladder rupture. Lymphopenia in combination with a monocytosis and neutrophilia was present in two dogs resulting in a classic stress leukogram. Both dogs were treated with corticosteroids previous to blood examination, which could account for this finding¹⁹. Biochemical blood analyses in other reports¹⁻⁸ have revealed several abnormalities common to dogs with a GM. These include high levels of ALT, ALP, GGT, BUN, total serum bilirubin concentration and bile acids. Especially high ALP, GGT and bilirubin concentrations are associated with decreased survival as they are assumed to reflect the severity of cholestasis¹³. Cholestasis may decrease clearance of bacteria by the liver, predisposing patients to infection and sepsis¹⁴. Other authors have found lower potassium in non-surviving dogs as compared to surviving dogs. They stated that hypokalemia likely reflected the more serious nature of a dog's illness, as it developed subsequent to chronic vomiting and bile peritonitis (third-space fluid accumulation)². Hypoproteinemia and hypoalbuminemia are associated with poor outcome in animals and humans with various conditions^{13,21,22}. Biochemical blood analyses in this study are mainly consistent with previous reports of elevated levels of ALP, ALT and bile acids and a hypoalbuminemia in the majority of dogs. Approximately one-third of the dogs had a hypokalemia and a hyponatremia. Hyperglycaemia is less frequently associated with GM, but was present in four out of seven dogs in this study. In two of these four dogs it was only mild (levels of 5.9 and 6.0 mmol/L) while the other two dogs had diabetes mellitus, which likely explains the hyperglycaemia in these cases. At our clinic GGT, BUN and total serum bilirubin concentrations are not routinely measured, so associations of these parameters with

GM cannot be compared to previous studies. None of the blood parameters were associated with reduced survival in this study, consistent with only one other report⁷.

Medical history, as well as physical and blood examinations may reveal abnormalities, but are mainly non-specific, and thus it can only be used as an indicative parameter of a GM. Definitive diagnosis is made via abdominal ultrasonography in most cases^{1,3,7}, which also applies for this study (22/23). Classic ultrasonographical presentation of a GM is immobile, echogenic bile with a striated or stellate pattern within the lumen of the gallbladder⁴. Presentation can, however, vary among dogs as they are at different stages of the disease process, and bile patterns are a suspected continuum³. In accordance with this, only four dogs in this study had a classic “kiwi fruit-like pattern” that is considered pathognomonic for GM and end-stage of the disease process^{3,4}. Interpretation of other bile patterns is challenging, as a stellate or striated pattern might not be observed in the early stages of a GM. Distinguishing these bile patterns from biliary sludge is difficult and in this study both were present at the same time in two dogs. Some authors have suggested that biliary sludge precedes the formation of a GM, and is therefore a continuous and not a separate process^{20,31}. Whether this is the case should be investigated with the use of a long term cohort study and multiple ultrasonographic follow up examinations. For now, to identify a GM, the lack of gravitational dependency appears to be of the utmost importance.

During ultrasonography, other aspects of the gallbladder were evaluated as well. Consistent enlargement of gallbladders as reported in previous studies³, did not appear in our study (12/23). Gallbladder wall thickness is variable in dogs with a GM³, and also in this study only five dogs had a thickened gallbladder wall. However, firmly establishing such thickening is difficult since exact measurements are not routinely reported. Moreover, reference ranges for normal and abnormal canine gallbladder volume^{3,23} and the normal range for wall thickness³ have not been well established, although the latter has been described as typically measuring 2-3mm²⁴. That these parameters are therefore highly susceptible to interpretation, is also suggested by the discrepancies between ultrasonographical and surgical findings in the present study. Three dogs had ultrasonographically as well as surgically detected enlarged gallbladders, two had ultrasonographically detected enlarged gallbladder that did not correspond with surgical findings and in three dogs the reverse was true. Similarly, detection of a thickened gallbladder during ultrasonography does not appear to be sensitive. A thickened gallbladder wall as was found during surgery in three dogs, was detected during ultrasonography in only one dog.

Gallbladder enlargement suggests biliary obstruction. In humans it is generally accepted that GM (also termed hydrops of the gallbladder) is a non-inflammatory condition resulting exclusively from obstruction of the cystic duct²⁶. Also veterinary research reports biliary obstruction to be highly associated with a GM^{1,2,3,11,13,28,29}. In this study, obstruction of the biliary tract, as detected upon surgery, was found to be associated with a GM, although not present in all dogs (11/13). Results of experimental studies^{2,31} indicate that enhanced exposure to bile acids (as a result of biliary stasis) increases the rate of gallbladder epithelial turnover and promotes the development of mucosal hyperplasia. Although cystic hyperplasia of the mucus glands in the wall of the gallbladder is a consistent pathological change associated with gallbladder mucocele, its aetiopathogenic role remains unproven¹. Terminology of the syndrome is confusing, and it has been referred to as mucinous hyperplasia, cystic hyperplasia of the gallbladder, mucinous cyst of the gallbladder, mucosal cysts of the gallbladder, cystic mucinous hypertrophy of the gallbladder mucosa, mucinous cholecystitis, and cystic glandular cholecystitis³⁷. In the present study twelve of the thirteen gallbladders examined showed signs of mucosal cystic hyperplasia. Although this supports an association between gallbladder mucosal cystic hyperplasia and GM formation, whether cystic hyperplasia of the gallbladder wall plays an aetiopathogenic role, is secondary to gallbladder dysfunction or dysmotility, or reflects a normal aging change, remains to be clarified. Since not all dogs had signs of biliary stasis, this is not likely to be the only causative factor in the formation of a GM.

Ultrasonographic findings are considered highly reliable for the identification of gallbladder rupture and include loss of gallbladder wall continuity, hyperechoic fat in the cranial portion of the abdomen, free abdominal fluid, and striated or stellate echogenic material outside the gallbladder lumen. Sensitivity of ultrasonography for gallbladder rupture is reported as high as 85.7%¹, which compares favourably to the reported sensitivity rate of 70.0% in humans³⁴. In this study, three out of four ultrasonographically detected gallbladder ruptures were confirmed during surgery (75%). Gallbladder rupture appears to result from physical distension of the gallbladder and resultant ischemic necrosis of the gallbladder wall, and should be considered a surgical emergency. Some authors suggest discretion to be used in determining the timing and necessity of surgery for dogs with an intact gallbladder, especially when clinical signs are present³. Recommendations have been made that surgery should be performed in all cases of ultrasonographical detection of a GM, irrespective of ultrasonographical findings of severity and gallbladder rupture^{6,13,25}. This is based on the fact

that there is a substantial risk of gallbladder rupture in dogs with a GM¹⁻³, and that GMs can be complicated by potentially fatal secondary bacterial infections^{4,5}. In this study, gallbladder rupture was only present in three out of twenty-three dogs, so risk of rupture seems to be lower than in other reports^{1,2}. However, dogs were presented at different disease stages and necrosis of the gallbladder wall was found during eight histopathological examinations (8/12). One could suggest that gallbladder rupture may have occurred in the future if surgery had not been performed. Nonetheless, this suggestion is not supported by a comparison of survival analyses.

EMST's between different treatment groups did not significantly differ. Although the EMST of the surgically treated group was 63.8 months as compared to 2.1 months in the group of dogs that received no treatment, this is most likely the result of a large number of censored cases (13/23). An important shortcoming of the Kaplan-Meier survival analysis is that dogs are not taken into account when they died of other causes or are still alive at follow up. However, not dying of a cause directly related to the GM can be considered a favourable clinical outcome and should be taken into account when considering different treatment possibilities. In the surgically treated group, mortality rate was high during the perioperative period (n=3, 23.1%) of which two had a gallbladder rupture. However, mortality was equally high after this period. Three dogs died at 1, 3 and 5 years later with clinical signs that suggested biliary tract disease. Only seven (53.8%) were alive at follow up (of which one had a gallbladder rupture at surgery) or had died of other causes. Furthermore, although gallbladder rupture is generally not associated with reduced survival^{1,2}, this study shows that prognosis is reserved despite surgical intervention, since two out of three dogs died. Of the dogs that received no or medical treatment, four dogs (40%) died within two months of diagnosis directly related to the GM. Dogs that survived this period were considered to have a very good prognosis, as none of these dogs (60%) died of a GM-related cause or were still alive at follow-up. Thus, performing surgery appears to increase the chance of survival, nor is always curative following the perioperative period. A possible explanation is that although the gallbladder itself is diseased, other parts of the biliary tract are malfunctioning as well. Removal of the gallbladder may not be sufficient to overcome these secondary problems. Decisions regarding treatment were mainly based upon the severity of clinical signs, and this may have introduced a bias in survival between different treatment groups. Less favourable clinical outcome in the surgically treated group may have solely been the result of the worse clinical status of this group. However, one would then expect a difference in survival between

the group of patients that underwent emergency cholecystectomy and the group of patients that underwent a more preventive cholecystectomy. Comparison of the EMST's between patients that underwent cholecystectomy with or without emergency indication did not yield a significant difference. Furthermore, no prognostic factors for survival (as a reflection of the severity of the disease) could be identified in this study. Both results make the existence of this bias highly unlikely.

Also, this study does not seem to support the assumption that a GM is a continuous disease process that can remain static for a period of time but will inevitably worsen². Results suggest that a GM can be an acute clinical condition and cause death in some dogs, but that it does not necessarily worsen in all dogs and may even spontaneously resolve. This possibility has been raised once before⁶. Why some dogs die of a GM (mostly acute) while others do not is an interesting question for further research. Follow-up information including ultrasonography may reveal differences in disease progression and the possibility of resolution. Environmental factors such as diet or concurrent diseases may play an aetiopathogenic role in the formation of a GM, and once under control, resolution of a GM might be possible. Unfortunately, it was not possible to investigate these parameters in the current study. It is therefore recommended that different treatment possibilities are thoroughly discussed with the owners. There is no argumentation as to which treatment is better, and decisions can only be based upon individual circumstances and personal preferences. Survival and clinical outcome after performing surgery may not always outweigh surgical risks, costs and the inevitable stress for dogs and owners.

The study reported here has several limitations. Firstly, because of the retrospective nature of the report, treatments and evaluations were not standardized and thus, data on these important factors were not always of the same quality or completeness. It is also impossible to determine whether environmental changes, control of concurrent diseases and different medical treatments would have influenced clinical outcomes. Possible aetiological factors can be associated with a GM, but a causative relation cannot be established retrospectively. Also, there were uncontrolled delays between some of the laboratory and imaging variables and the surgical or necropsy confirmation of the gallbladder status. Monitoring of disease progression was not standardized between dogs and could further vary as certain parameters were measured at different intervals. Another limitation of this study is the reliability of the follow-up information, since necropsies were not performed consistently in dogs that died of causes related or unrelated to biliary tract disease in the long term. Decisions regarding cause of

death were therefore solely based on verbal information from owners and referring veterinarians. Another bias was introduced because some dogs with potentially manageable biliary complications may have been euthanized because of financial constraints. In these types of studies, the issue of how to classify dogs that have been euthanized is controversial. Two other methods of dealing with this problem have been proposed¹³, exclusion of all animals that were euthanized and classification of all dogs that were euthanized as having died of causes unrelated to the underlying disease. Both of these methods are valid, but have the disadvantage of decreasing the number of animals included in the study and of disregarding the likelihood that animals were euthanized because of the GM. In the present study, few patients were included (n=23) which already resulted in difficulties to establish statistically significant associations, and in broadened confidence intervals. Excluding all euthanized patients, or counting them as censored cases, would have left only nine cases to investigate and was therefore considered an unsuitable approach.

The purpose of this study was first to describe signalment, medical history, ultrasonographical, clinical- and histopathological findings of dogs diagnosed with GM in the Dutch referral population. Secondly, to assess outcome and survival after diagnosis and to identify prognostic factors observed at initial presentation for reduced survival times. A retrospective study design is sufficient to achieve these goals. However, answering some questions usually raises other questions, as in this study. Underlying aetiopathogenesis and factors influencing disease progression remains to be clarified. Prospective trials with a larger number of animals, a control-group, and with standardised treatments and follow-up monitoring are likely essential for such clarification and recommended for further research.

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