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Percutaneous ultrasound-guided cholecystocentesis: Complications and association of ultrasonographic findings with bile culture results.

Structured Summary

Objectives: To retrospectively evaluate cases presented for percutaneous ultrasound-guided cholecystocentesis (PUC) for associated complications, identify any risk factors associated with complications and to assess ultrasonographic findings and relate these to bacterial culture results.

Methods: Data of 300 patients presented for PUC were retrospectively collected (signalment, number of PUC performed, bile culture results, cytology results, ultrasonographic findings, complications and outcome) and ultrasonographic images assessed for defined structural changes. The prevalence of major complications was determined with 95% confidence intervals (95% CI). Multivariable multilevel logistic regression was used to determine association of ultrasonographic findings with a positive bile culture.

Results: 300 PUC procedures performed in 201 dogs and 51 cats were included; 35 patients had the procedure performed more than once. The overall prevalence of major complications was 8/300 procedures (2.7%; 95% CI 1.4-5.2%). The prevalence of bile peritonitis was 2/300 procedures (0.7%; 95%CI 0.2-2.4%). An abnormal appearance on ultrasound examination was found in 52% of cases. An ultrasonographically abnormal gall bladder showed a sensitivity, specificity and accuracy of 82%, 55.7% and 61.5%, respectively, to predict a positive bile culture. Significant associations with a positive bile culture were confirmed only for maximum wall thickness and an irregular luminal surface.

Clinical significance: PUC is overall a safe technique, if carried out in selected patients. Abnormal ultrasonographic findings are only a fair predictor of a positive bile culture.

Keywords: Canine, feline, ultrasound, bile, complication

Introduction:

Ultrasonographic assessment of the gall bladder and percutaneous ultrasound-guided cholecystocentesis (PUC) are commonly performed procedures in the assessment of hepatobiliary diseases. Their diagnostic value is the ability to assess for structural abnormalities and obtain samples for culture and cytology examinations. The technique and feasibility of PUC has been described in several species including dogs, cats, cows and man (Braun & Gerber 1992, Savary-Bataille *et al.* 2003, Tudyka *et al.* 1995, Voros *et al.* 2002). Positive bacterial culture results and abnormal cytology in dogs and cats investigated for hepatobiliary disease are relatively common findings and are likely to impact on case management (Wagner *et al.* 2007). The use of PUC is viewed by some clinicians as a procedure with undefined risk of serious complications, most importantly bile peritonitis. Complications associated with this procedure have been reported in human patients and animals, including bile leakage and bile peritonitis, haemorrhage, haemobilia and bacteraemia/sepsis and even fatal vaso-vagal reactions (Center 2009, Nyland *et al.* 2015, Peters *et al.* 2016).

Ultrasonographic findings have been reported for several gall bladder or biliary pathologies (e.g. biliary mucocele and cholecystitis) (Brain *et al.* 2006, Crews *et al.* 2009, DeMonaco *et al.* 2016). Signs commonly reported with septic cholecystitis and cholangitis/cholangiohepatitis are symmetric or asymmetric gall bladder wall thickening, dilated common bile duct, double layered wall, hyperechoic wall, hyperechoic gall bladder contents and choleliths (Center 2009, Gaschen 2009, Hittmair *et al.* 2001, Nyland *et al.* 2015).

To the authors' knowledge the veterinary literature includes no information on the risk of major complications associated with PUC or the ultrasonographic findings associated with the presence of bacteria within bile (positive bacterial culture).

The aim of this study was to retrospectively evaluate cases presented for percutaneous ultrasound-guided cholecystocentesis for associated complications, and to describe and determine the prevalence of encountered complications. Secondary aims were to identify any risk factors associated with complication and to assess ultrasonographic findings and relate these to bacterial culture results. We hypothesised that the prevalence of complications is low and positive bile culture result cannot be predicted from ultrasonographic findings.

Material and Methods

The patient database of the University of Liverpool was searched for percutaneous ultrasound-guided cholecystocentesis procedures performed between January 2009 and March 2016. Approval was granted by the Committee on Research Ethics at the University of Liverpool (VREC452). Documented performance of PUC was the main inclusion criterion. For each case hospitalisation records, client and referring veterinary surgeon communication documentation, laboratory data records and ultrasonographic reports were examined. Data obtained from the clinical records included signalment, history, number of PUC performed on different occasions, immunosuppressive treatment, bile culture (y/n), culture results and isolated bacteria, cytology (y/n), cytology results, ultrasonographic findings and complications and their outcome. Complications were defined as a major adverse event (e.g. clinical deterioration, death) in close temporal relation (3 days) to the PUC. Self-limiting events (e.g. minor amount of peritoneal fluid post PUC, mild abdominal pain on palpation) were not recorded as complication as these were considered to be less consistently recorded and did not influence the final outcome.

All ultrasound examinations were performed by a veterinary radiologist, a radiology resident or an experienced ultrasonographer with either a LOGIQ 7 or a LOGIQ S8 (General Electric Medical System,

Milwaukee, Wisconsin) or a Z.one (Zonare Medical Systems, Mountain View, California). Most commonly, a curvilinear microconvex probe (8 to 11 MHz) was used for assessment and during the PUC procedure. All PUC procedures were performed under sedation or general anaesthesia by a veterinary radiologist or a radiology resident. The anaesthetic protocol was chosen by the attending anaesthetist or clinician and constant monitoring was provided for all cases.

Available ultrasonographic images of the gall bladder were reviewed, where available, by the first author and the following parameters assessed: mean thickness of the gall bladder wall measured in three locations (excluding the neck of the gall bladder), maximum wall thickness, irregularity of the wall thickness, presence of a double rim pattern, presence of irregular luminal surface (Figure 1), presence of diffusely hyperechoic wall, pattern of gall bladder contents (normal [anechoic], echoic sediment, anechoic rim along the inner margin, anechoic fragments within echoic sediment, stellate pattern, kiwi-fruit-like pattern, hyperechoic sediment, cholelith, or empty. Measurements were conducted using Visbion image viewer software (Visbion Ltd., Chertsey, UK). Two groups were subsequently defined as having either normal or abnormal ultrasonographic findings related to the gall bladder. Abnormal was defined as one or more of the following parameters: mean wall thickness more than 2 mm (dog) (Nyland *et al.* 2015) or more than 1mm (cat) (Hittmair *et al.* 2001), irregular wall thickness, double rim pattern, irregular luminal surface, diffusely hyperechoic wall, gall bladder contents other than anechoic contents or echoic sediment.

Statistical analysis

Statistical analyses were performed with the computer programs SPSS 22.0 (SPSS Inc., Chicago, Illinois, USA), MLwiN (Version 2.20, Centre for Multilevel Modelling, University of Bristol) and R (R version 3.2.0, The R Foundation for Statistical Computing). Independent variables were derived from information obtained from the signalment data, clinical records, ultrasound reports and archived images, and patient follow-up. Variables assessed included those related to the animal (species, weight, sex, age, breed, presenting complaint), number of cholecystocentesis procedures

performed, any immunosuppressive treatment and ultrasound parameters assessed (gall bladder wall thickness, irregularity of gall bladder wall, presence of irregular luminal surface, presence of hyperechoic gall bladder wall, presence of gall bladder wall oedema, gall bladder contents).

Descriptive statistics were calculated; continuous data summarised as median values with interquartile ranges, and categorical data expressed as frequencies with 95% confidence intervals (95% CI). For categorical variables with many categories and/or categories containing only small numbers, categories were combined into appropriate larger classes. For continuous variables (age, weight, gall bladder wall thickness and number of cholecystocentesis performed), the functional form (shape) of the variable with respect to the outcome was assessed using generalised additive models (GAM) fitted using cubic spline smoothers. Normality of distribution for continuous variables was assessed using the Kolmogorov-Smirnov test.

Cholecystocentesis procedures were considered the unit of interest, the binary outcome for each was the presence or absence of a complication following the procedure. Obtaining a positive culture result was considered as an additional outcome, with this analysis restricted to the ultrasonographic variables identified. As many animals underwent multiple procedures, these were clustered within animals and to account for this, factors affecting the occurrence of complications or positive culture were examined using multilevel, multivariable logistic regression models. Within-animal clustering of cholecystocentesis procedures was accounted for as a random intercept term in all models. All variables that showed some association on initial univariable analysis (a *P*-value <0.25) were considered for incorporation into a final multivariable model and for variables with a correlation coefficient of $\geq$0.70, the variable with the smallest *P*-value was considered for further analysis. The model was constructed by a manual backwards stepwise procedure where variables with Wald *P*-values < 0.05 were retained in the model.

Results

Population

A total of 300 PUC procedures were performed in 201 dogs and 51 cats. The procedure was performed more than once in 35 (13.9%) patients. PUC was performed once in 217 patients (86.1%; 176 dogs and 41 cats), twice in 26 patients (10.3%; 17 dogs and 9 cats), three times in 6 dogs (2.4%), four times in 2 dogs (0.8%) and five times in one cat (0.4%).

Of 201 dogs, 12 were female entire, 91 female neutered, 31 male entire, and 67 male neutered. Their ages ranged from 7 months to 175 months (median age 91 months). The body weight ranged from 2.2 to 66.1kg (median 14.5 kg).

Of 51 cats, 16 were female neutered and 35 were male neutered. Their ages ranged from 13 months to 192 months (median age 107 months). The body weight ranged from 2.1 to 6.3 kg (median 3.8 kg).

Fifty-two breeds were represented in the canine population; the most common were Cross Breed (24), English Springer Spaniel (16), Labrador Retriever (14) and Miniature Schnauzer and Cocker Spaniel (both 13). Ten breeds were represented in the feline population; the most common cat breeds were Domestic Shorthair (30), British Shorthair (6) and Domestic Longhair (4).

For 271/300 (90.3%) PUC procedures a follow-up of more than 24hrs was documented. Four patients died within the 24hr period.

Complications

The overall prevalence of complications was 8 of 300 cholecystocentesis attempts (2.7%; 95% CI 1.4-5.2%). The prevalence of bile peritonitis was 2 of 300 procedures (0.7%; 95%CI 0.2-2.4%). These complications occurred in 5 canine and 3 feline patients (Table 1). Two dogs developed bile peritonitis. This was confirmed by aspiration of bilious fluid and ultrasonographic signs of peritonitis in one case and by exploratory laparotomy in the other. The first patient had persistent septic cholecystitis and after the third PUC in the course of the disease the dog developed bile peritonitis. Ultrasound assessment revealed a double-rim pattern and wall thickening at the time of the third PUC. The second dog was diagnosed ultrasonographically with a biliary mucocele and a PUC was

performed on day one. Exploratory laparotomy was performed on day three due to clinical deterioration at which time there was peritoneal fluid and macroscopic evidence of adhesion to the gall bladder. The dog died one day after surgery.

Three patients (one dog and two cats) suffered cardiorespiratory arrest and did not recover from anaesthesia after the PUC. One of these dogs had an early mucocele diagnosed ultrasonographically. One dog developed systemic inflammatory response syndrome within 24hrs of PUC was treated medically and survived, one cat developed acute kidney injury and was euthanized, and one dog developed hypotensive shock one day post procedure and died. None of these patients had ultrasonographic evidence of peritonitis or haemorrhage.

Bile culture and cytology

Bile culture was performed in 294 cases and 64 (21.3%; 95% CI 17.1-26.3%) produced positive results. Most common isolates were *Escherichia coli* (41/64), *Enterococcus* spp. (22/64), *Clostridium perfringens* (5/64), *Bacteroides* spp. (2/64) and *Actinomyces* spp. (2/64). *Lactobacillus* spp., *Lactococcus* spp., *Listeria* spp., *Klebsiella* spp., *Salmonella* sp., *Streptococcus bovis* and *Pseudomonas* spp. were all isolated once. Mixed populations were identified in 28/64 samples. Bile cytology was performed in 201 cases and 35 (17.3%) showed bactibilia, 25 (12.4%) increased mucus contents, 7 (3.5%) inflammatory cells, and 134 showed no significant findings.

For the 64 cases with a positive bile culture, 37 (57.8%) also had cytology results available. 30/37 (81.1%) had bacteria seen on cytological examination. In four cases with a negative bile culture there were bacteria present on cytological examination.

All patients with complications had bile culture results available. 3/8 dogs with complications had positive bile culture, 1/3 developed bile peritonitis. Only 1/6 dogs with evidence of an early or mature mucocele had a positive bile culture result.

Ultrasonographic findings

A total of 294/300 PUC had adequate ultrasound images available for reassessment. An abnormal appearance of the gall bladder was found in 154/294 cases. The mean wall thickness for normal gall bladders was 1.3mm in dog and 0.8mm in cats. For gall bladders categorized as abnormal the mean wall thickness range was 0.8-11.2mm (mean 2.2mm) in dogs; in cats the mean wall thickness range was 0.5-4.8mm (mean 1.6mm). In 95 cases (61.7%) there was an increased mean wall thickness. In 73 cases (47.4%) the gall bladder wall was found to be irregular in thickness and an irregular luminal surface was found in 107 cases (69.5%) (Figure 1). A double rim pattern was found in 8 cases (2.7%) and a diffusely hyperechoic wall in 33 cases (11.2%). Normal or echoic biliary sediment was present in 265 gall bladders (90.1%). Hyperechoic sediment was seen in 10 cases (3.4%) and gall bladder cholelithsin 13 cases (4.4%). Signs of an early or mature mucocele were present in 6 patients (2.0%). Bile culture results and ultrasonographic images were available for review in 289 cases. Of these 151 had abnormal ultrasonographic findings; 50/289 had abnormal findings and a positive bile culture result. A positive bile culture result with a normal appearance on ultrasound was seen in 11/289 patients. However, no evidence of bacteria was seen in 101 cases with ultrasonographically abnormal gall bladders. A sensitivity of 82%, specificity of 55.7% and accuracy of 61.5% was determined for abnormal ultrasound findings to predict positive bile culture results.

Associations between independent variables and positive culture

The number of complications was deemed insufficient for meaningful statistical analysis and so only having a positive bile culture was considered as an outcome for the multivariable analysis. None of the continuous variables demonstrated a significantly non-linear relationship with the outcome considered and so all were incorporated into the subsequent logistic regression analyses.

Maximum gall bladder wall thickness, irregular gall bladder wall thickness, irregular gall bladder luminal surface, hyperechoic gall bladder wall and gall bladder contents all demonstrated some

association with a positive culture on univariable analysis, with only maximum wall thickness and an irregular luminal surface remaining significant on multivariable analysis (Table 2).

Discussion

The prevalence of complications specifically following percutaneous ultrasound-guided cholecystocentesis has to the authors' knowledge not been investigated. PUC is an important diagnostic technique to assess the hepatobiliary system and can be associated with major complications, such as bile peritonitis. In the present study a prevalence of major complications of 2.7% (95% CI 1.4-5.2%) and for bile peritonitis of 0.7% (95%CI 0.2-2.4%) was determined. The procedure can therefore be considered as reasonably safe.

Of the eight patients with complications only two developed confirmed bile peritonitis. These were most likely caused or potentiated by the PUC procedure. The other six cases developed other forms of major complications, e.g. AKI and SIRS or did not recover from anaesthesia. Although an association with the performed PUC cannot be excluded, there was no direct evidence for this and other procedures (e.g. hepatic biopsies) and the concurrent pathology could also have been responsible for the complication.

The study was conducted retrospectively and therefore there was a selection bias towards cases that were considered by the radiologist and attending clinician to be safe to sample. Individual experience and the clinical expectation of the diagnostic utility of the sample will have contributed to this decision. It is therefore important to note that cases with certain gall bladder changes (e.g. emphysematous cholecystitis) or other conditions (e.g. severe coagulopathies) were not sampled and therefore not included in this study. A prospective study would be necessary to assess the prevalence of complications without any such bias, but would not be ethical to conduct. Nonetheless, in a clinical situation it is likely that experienced clinicians would make similar judgements to those made at our institution and therefore a comparable prevalence of complications might be expected.

Six cases of early and mature biliary mucoceles were diagnosed ultrasonographically and were sampled via PUC. Two of these cases developed complications after PUC. A biliary mucocele is stated by some authors as a contraindication for PUC, as it is often associated with gall bladder wall necrosis and PUC can therefore facilitate rupture of the gall bladder (Nyland *et al.* 2015). However, Besso *et al.* (2000) suggested sampling of all mucoceles to assess for infection. A decision, as to whether sampling can be conducted safely, needs to be considered very carefully in cases of biliary mucoceles. Considering that only 1/6 dogs with early or mature mucoceles had a positive bile culture, it is questionable that the benefit outweighs the risk of PUC and sampling, especially of mature mucoceles, should probably be avoided.

In the veterinary literature, studies investigating other aspects of hepatobiliary diagnostics or disease comment on complications associated with cholecystocentesis, although often this was conducted during surgery. Brain *et al.* (2006) reported in their case series of feline cholecystitis that one of four cats developed bile peritonitis after PUC. In contrast, Peters *et al.* (2016) in their paper on the diagnostic utility of cytological assessment of bile aspirates describe that of 140 patients, complications were seen in four dogs and one cat. Only three of these were seen after PUC. However, this study does not clarify how many aspirates in total were obtained with ultrasound-guidance. Studies performed in healthy cats and dogs and dogs treated with hydrocortisone have shown a similarly low prevalence of complications (Kook *et al.* 2010, Savary-Bataille *et al.* 2003, Voros *et al.* 2002). However, the patient number in each of these studies was small. Furthermore, it is likely that the prevalence of complications differs between a healthy population and patients with hepatobiliary or other diseases. The results of these studies still suggest a low prevalence of complications, which is reflected in the results of the present study.

It should be noted that two of eight patients with complications had no ultrasonographic abnormalities and in those with abnormalities the findings were often subtle. The statistical analysis also failed to demonstrate an association between structural gall bladder wall changes and

complications. These findings indicate that there is no predictable relationship between the severity of gall bladder wall changes and the risk of complications.

A documented follow-up period of 24hr or more was not present in 9.7% of the assessed cases. It is unlikely that patients with major complication neither re-presented nor the hospital notified by the owner or referring veterinary surgeon about major complications. However, it remains possible that major complications were missed in these patients. As mentioned above, it remains unknown whether complications could have developed in those cases for which the radiologist elected not to perform a PUC. The results of this study should be interpreted with this potential selection bias in mind.

Consistent with other studies, we found a positive bacterial culture in 21.3% of samples (Crews *et al.* 2009, Peters *et al.* 2016). In contrast to the study of Peters *et al.* (2016), bacteria were found less frequently (17.3%) during cytological examination. Only four cases were identified with a negative culture, but bacteria on cytology. This may be secondary to interference of previous or concurrent treatment with antimicrobials. The most common isolates (*E. coli, Enterococcus* spp.) were also found to be similar to other studies in the veterinary literature (Brain *et al.* 2006, Kook *et al.* 2010, Peters *et al.* 2016, Tamborini *et al.* 2016, Wagner *et al.* 2007).

The sensitivity (82%), specificity (55.7%) and accuracy (61.5%) of abnormal ultrasound findings to predict a positive culture result was only fair. However, an association of positive bile culture and increased wall thickness on ultrasound and/or irregular luminal surface was seen. Both of these features are commonly encountered with septic cholecystitis (Brain *et al.* 2006, Nyland *et al.* 2015, Tamborini *et al.* 2016). In humans, cats and dogs, an association between cholelithiasis and bacterial infection has been reported (Eich & Ludwig 2002, Kirpensteijn *et al.* 1993, Tabata & Nakayama 1981). This could not be demonstrated in the present study. Ultrasonographic abnormalities should therefore be considered of only limited value in the decision-making process for obtaining a PUC. However, an increased wall thickness and/or irregular luminal surface may especially warrant aspiration of bile.

The study has several limitations, most of which are associated with its retrospective nature. As mentioned previously the population in this study is likely biased as certain gall bladder or concurrent pathologies will have led to the decision at the time not to perform a PUC. However, this does reflect the real situation seen in clinical practice. An appropriate patient selection is necessary to keep the prevalence of complications as low as determined in the present study.

The retrospective assessment of ultrasonographic images assumes that the saved images are representative for the case. Ultrasound remains a highly user dependent modality and image interpretation is to some degree subjective, even in prospective studies. However, it is the imaging modality of choice to assess the biliary system.

It was not possible to confirm PUC as the true and only cause for complications in several cases. A post mortem examination was not carried out in any of the cases with complications. The cases with bile peritonitis are possibly the least controversial. However, a causative association cannot be ruled out in any case and a marginal overestimate in the prevalence of complications is more appropriate for patient safety in this context. Due to the multiple factors considered, detection of spurious associations with the finding of a positive bile culture is a possibility; however, the factors identified are biologically plausible.

In conclusion, percutaneous ultrasound-guided cholecystocentesis was confirmed to be a safe technique, if carried out on appropriately selected patients. There might be an increased risk with the presence of a biliary mucocele. Abnormal ultrasonographic findings are only a fair predictor of a positive bile culture. However, in cases with increased gall bladder wall thickness and/or an irregular luminal surface sampling may be warranted.

No conflicts of interest have been declared.

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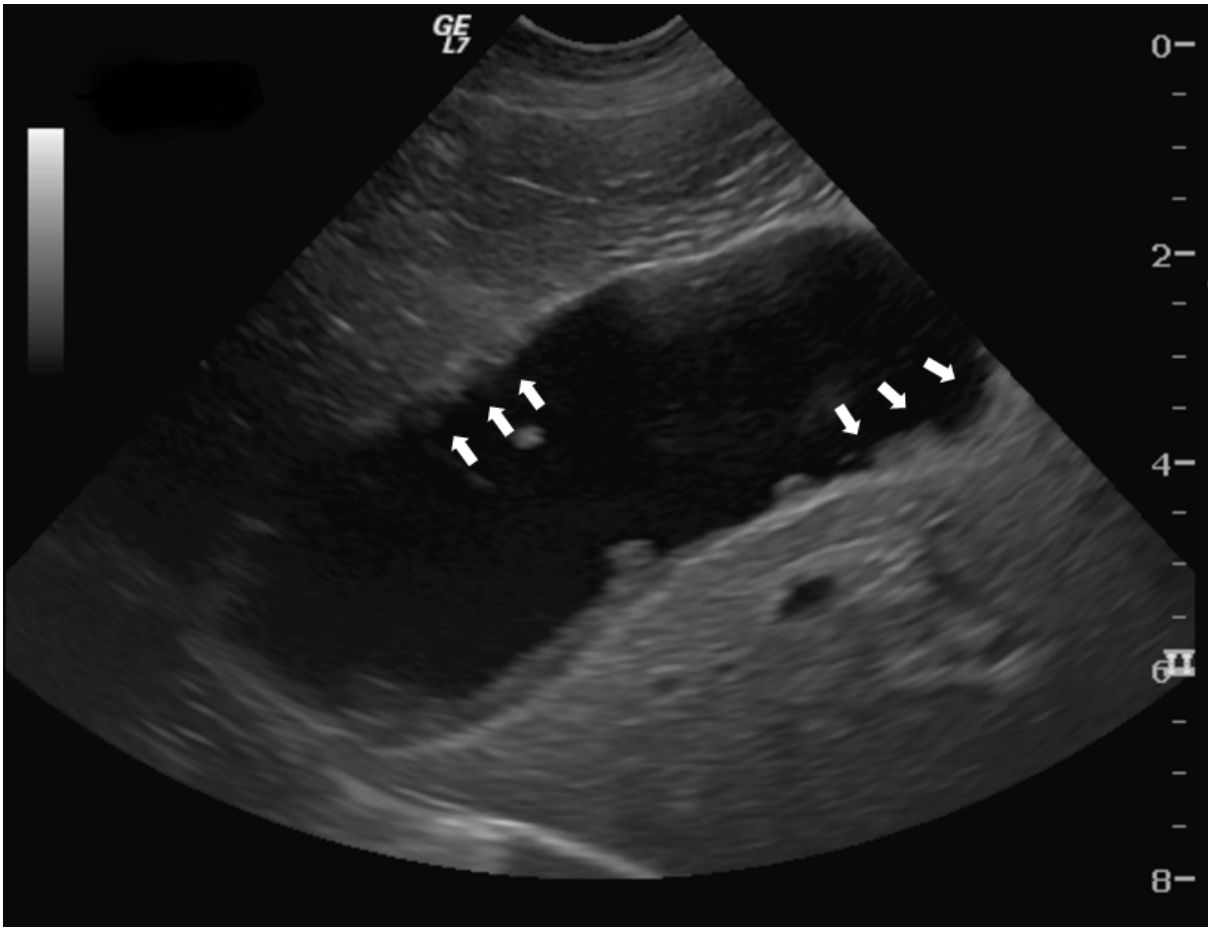

Figure 1 – Longitudinal ultrasonographic view of the gall bladder of a dog. Note the irregular luminal surface (arrows) and irregularly increased wall thickness.

Table 1 **Complications, outcome and ultrasonographic findings following 300 percutaneous ultrasound-guided cholecystocentesis procedures in 201 dogs and 51 cats**

	Breed	Diagnosis	Complication	Outcome	Ultrasonographic findings
1	Border Terrier	Biliary mucocele	Bile peritonitis	Died	Mature biliary mucocele
2	Flat Coated Retriever	Chronic septic cholecystitis	Bile peritonitis	Euthanasia	Thickened gall bladder wall (mean 2.4mm) and double rim pattern
3	Border Collie	Septic cholecystitis (concurrent controlled IMHA/IMTP)	SIRS	Survived to discharge after medical treatment	Hyperechoic sediment
4	Soft Coated Wheaten Terrier	Septic cholecystitis (concurrent meningioma)	Hypotensive shock	Died	Normal
5	Siamese	Granulocytic hypoplasia	AKI	Died	Increased wall thickness (mean 1.2mm)

6	DSH	Chronic hepatitis	Cardiorespiratory arrest during anaesthesia	Died	Increased and irregular wall thickness (mean 1.5mm), irregular luminal surface
7	WHWT	Lymphoma	Cardiorespiratory arrest during anaesthesia	Died	Increased and irregular wall thickness (mean 2.6mm), anechoic inner rim (early mucocele), irregular luminal surface
8	Persian	Open final diagnosis	Cardiorespiratory arrest during anaesthesia	Died	Normal

AKI acute kidney injury, *DSH* domestic short hair cat, *IMHA* immune-mediated haemolytic anaemia, *IMTP* immune-mediated thrombocytopenia, *SIRS* systemic inflammatory response syndrome, *WHWT* West Highland White Terrier

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Table 2 Results of multilevel, multivariable analyses for associations with positive bile culture following 300 percutaneous ultrasound-guided cholecystocentesis procedures in 201 dogs and 51 cats

Outcome	Variable	Category	OR	95% CI	*P*-value
Positive culture	**Maximum gall bladder wall thickness**	(mm)	1.47	1.04-2.08	**0.029**
	Irregular luminal surface	No	(Ref)	-	-
		Yes	2.97	1.45-6.08	**<0.001**

P-values are from the Wald chi-squared test; *CI,* confidence interval; *OR*, odds ratio;

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