1 Word count: 4193

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

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### 5 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.

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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% Cl). Multivariable multilevel logistic regression was used to determine association of ultrasonographic findings with a positive bile culture.

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17 **Results:** 300 PUC procedures performed in 201 dogs and 51 cats were included; 35 patients had the 18 procedure performed more than once. The overall prevalence of major complications was 8/300 19 procedures (2.7%; 95% CI 1.4-5.2%). The prevalence of bile peritonitis was 2/300 procedures (0.7%; 20 95%CI 0.2-2.4%). An abnormal appearance on ultrasound examination was found in 52% of cases. An 21 ultrasonographically abnormal gall bladder showed a sensitivity, specificity and accuracy of 82%, 22 55.7% and 61.5%, respectively, to predict a positive bile culture. Significant associations with a 23 positive bile culture were confirmed only for maximum wall thickness and an irregular luminal 24 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.
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28 Keywords: Canine, feline, ultrasound, bile, complication

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- 30 Introduction:

31 Ultrasonographic assessment of the gall bladder and percutaneous ultrasound-guided 32 cholecystocentesis (PUC) are commonly performed procedures in the assessment of hepatobiliary 33 diseases. Their diagnostic value is the ability to assess for structural abnormalities and obtain 34 samples for culture and cytology examinations. The technique and feasibility of PUC has been 35 described in several species including dogs, cats, cows and man (Braun & Gerber 1992, Savary-36 Bataille et al. 2003, Tudyka et al. 1995, Voros et al. 2002). Positive bacterial culture results and 37 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 38 39 by some clinicians as a procedure with undefined risk of serious complications, most importantly bile 40 peritonitis. Complications associated with this procedure have been reported in human patients and 41 animals, including bile leakage and bile peritonitis, haemorrhage, haemobilia and 42 bacteraemia/sepsis and even fatal vaso-vagal reactions (Center 2009, Nyland et al. 2015, Peters et 43 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).

50 To the authors' knowledge the veterinary literature includes no information on the risk of major 51 complications associated with PUC or the ultrasonographic findings associated with the presence of 52 bacteria within bile (positive bacterial culture).

The aim of this study was to retrospectively evaluate cases presented for percutaneous ultrasoundguided 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.

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#### 60 Material and Methods

61 The patient database of the University of Liverpool was searched for percutaneous ultrasound-62 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). 63 64 Documented performance of PUC was the main inclusion criterion. For each case hospitalisation 65 records, client and referring veterinary surgeon communication documentation, laboratory data 66 records and ultrasonographic reports were examined. Data obtained from the clinical records included signalment, history, number of PUC performed on different occasions, immunosuppressive 67 treatment, bile culture (y/n), culture results and isolated bacteria, cytology (y/n), cytology results, 68 69 ultrasonographic findings and complications and their outcome. Complications were defined as a 70 major adverse event (e.g. clinical deterioration, death) in close temporal relation (3 days) to the PUC. 71 Self-limiting events (e.g. minor amount of peritoneal fluid post PUC, mild abdominal pain on 72 palpation) were not recorded as complication as these were considered to be less consistently 73 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.

81 Available ultrasonographic images of the gall bladder were reviewed, where available, by the first 82 author and the following parameters assessed: mean thickness of the gall bladder wall measured in 83 three locations (excluding the neck of the gall bladder), maximum wall thickness, irregularity of the 84 wall thickness, presence of a double rim pattern, presence of irregular luminal surface (Figure 1), 85 presence of diffusely hyperechoic wall, pattern of gall bladder contents (normal [anechoic], echoic 86 sediment, anechoic rim along the inner margin, anechoic fragments within echoic sediment, stellate 87 pattern, kiwi-fruit-like pattern, hyperechoic sediment, cholelith, or empty. Measurements were 88 conducted using Visbion image viewer software (Visbion Ltd., Chertsey, UK). Two groups were 89 subsequently defined as having either normal or abnormal ultrasonographic findings related to the 90 gall bladder. Abnormal was defined as one or more of the following parameters: mean wall thickness 91 more than 2 mm (dog) (Nyland et al. 2015) or more than 1mm (cat) (Hittmair et al. 2001), irregular 92 wall thickness, double rim pattern, irregular luminal surface, diffusely hyperechoic wall, gall bladder 93 contents other than anechoic contents or echoic sediment.

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#### 95 Statistical analysis

96 Statistical analyses were performed with the computer programs SPSS 22.0 (SPSS Inc., Chicago, 97 Illinois, USA), MLwiN (Version 2.20, Centre for Multilevel Modelling, University of Bristol) and R (R 98 version 3.2.0, The R Foundation for Statistical Computing). Independent variables were derived from 99 information obtained from the signalment data, clinical records, ultrasound reports and archived 100 images, and patient follow-up. Variables assessed included those related to the animal (species, 101 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).

105 Descriptive statistics were calculated; continuous data summarised as median values with 106 interquartile ranges, and categorical data expressed as frequencies with 95% confidence intervals 107 (95% CI). For categorical variables with many categories and/or categories containing only small 108 numbers, categories were combined into appropriate larger classes. For continuous variables (age, 109 weight, gall bladder wall thickness and number of cholecystocentesis performed), the functional 110 form (shape) of the variable with respect to the outcome was assessed using generalised additive 111 models (GAM) fitted using cubic spline smoothers. Normality of distribution for continuous variables 112 was assessed using the Kolmogorov-Smirnov test.

113 Cholecystocentesis procedures were considered the unit of interest, the binary outcome for each 114 was the presence or absence of a complication following the procedure. Obtaining a positive culture 115 result was considered as an additional outcome, with this analysis restricted to the ultrasonographic 116 variables identified. As many animals underwent multiple procedures, these were clustered within 117 animals and to account for this, factors affecting the occurrence of complications or positive culture 118 were examined using multilevel, multivariable logistic regression models. Within-animal clustering of 119 cholecystocentesis procedures was accounted for as a random intercept term in all models. All 120 variables that showed some association on initial univariable analysis (a P-value <0.25) were 121 considered for incorporation into a final multivariable model and for variables with a correlation coefficient of >0.70, the variable with the smallest P-value was considered for further analysis. The 122 123 model was constructed by a manual backwards stepwise procedure where variables with Wald P-124 values < 0.05 were retained in the model.

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126 Results

127 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

134 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.

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145 Complications

146 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%Cl 0.2-2.4%). These 147 complications occurred in 5 canine and 3 feline patients (Table 1). Two dogs developed bile 148 149 peritonitis. This was confirmed by aspiration of bilious fluid and ultrasonographic signs of peritonitis 150 in one case and by exploratory laparotomy in the other. The first patient had persistent septic 151 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 152 153 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.

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### 164 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.

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179 Ultrasonographic findings

180 A total of 294/300 PUC had adequate ultrasound images available for reassessment. An abnormal 181 appearance of the gall bladder was found in 154/294 cases. The mean wall thickness for normal gall 182 bladders was 1.3mm in dog and 0.8mm in cats. For gall bladders categorized as abnormal the mean 183 wall thickness range was 0.8-11.2mm (mean 2.2mm) in dogs; in cats the mean wall thickness range 184 was 0.5-4.8mm (mean 1.6mm). In 95 cases (61.7%) there was an increased mean wall thickness. In 185 73 cases (47.4%) the gall bladder wall was found to be irregular in thickness and an irregular luminal 186 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 187 188 in 265 gall bladders (90.1%). Hyperechoic sediment was seen in 10 cases (3.4%) and gall bladder 189 choleliths in 13 cases (4.4%). Signs of an early or mature mucocele were present in 6 patients (2.0%). 190 Bile culture results and ultrasonographic images were available for review in 289 cases. Of these 151 191 had abnormal ultrasonographic findings; 50/289 had abnormal findings and a positive bile culture 192 result. A positive bile culture result with a normal appearance on ultrasound was seen in 11/289 193 patients. However, no evidence of bacteria was seen in 101 cases with ultrasonographically 194 abnormal gall bladders. A sensitivity of 82%, specificity of 55.7% and accuracy of 61.5% was 195 determined for abnormal ultrasound findings to predict positive bile culture results.

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197 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.

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203 Maximum gall bladder wall thickness, irregular gall bladder wall thickness, irregular gall bladder 204 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).

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## 208 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.

221 The study was conducted retrospectively and therefore there was a selection bias towards cases that 222 were considered by the radiologist and attending clinician to be safe to sample. Individual 223 experience and the clinical expectation of the diagnostic utility of the sample will have contributed 224 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 225 226 and therefore not included in this study. A prospective study would be necessary to assess the 227 prevalence of complications without any such bias, but would not be ethical to conduct. 228 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 229 230 complications might be expected.

231 Six cases of early and mature biliary mucoceles were diagnosed ultrasonographically and were 232 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 233 234 necrosis and PUC can therefore facilitate rupture of the gall bladder (Nyland et al. 2015). However, 235 Besso et al. (2000) suggested sampling of all mucoceles to assess for infection. A decision, as to 236 whether sampling can be conducted safely, needs to be considered very carefully in cases of biliary 237 mucoceles. Considering that only 1/6 dogs with early or mature mucoceles had a positive bile 238 culture, it is questionable that the benefit outweighs the risk of PUC and sampling, especially of 239 mature mucoceles, should probably be avoided.

240 In the veterinary literature, studies investigating other aspects of hepatobiliary diagnostics or 241 disease comment on complications associated with cholecystocentesis, although often this was 242 conducted during surgery. Brain et al. (2006) reported in their case series of feline cholecystitis that 243 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, 244 245 complications were seen in four dogs and one cat. Only three of these were seen after PUC. 246 However, this study does not clarify how many aspirates in total were obtained with ultrasound-247 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, 248 249 Voros et al. 2002). However, the patient number in each of these studies was small. Furthermore, it 250 is likely that the prevalence of complications differs between a healthy population and patients with 251 hepatobiliary or other diseases. The results of these studies still suggest a low prevalence of 252 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 severityof 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).

272 The sensitivity (82%), specificity (55.7%) and accuracy (61.5%) of abnormal ultrasound findings to 273 predict a positive culture result was only fair. However, an association of positive bile culture and 274 increased wall thickness on ultrasound and/or irregular luminal surface was seen. Both of these 275 features are commonly encountered with septic cholecystitis (Brain et al. 2006, Nyland et al. 2015, 276 Tamborini et al. 2016). In humans, cats and dogs, an association between cholelithiasis and bacterial 277 infection has been reported (Eich & Ludwig 2002, Kirpensteijn et al. 1993, Tabata & Nakayama 278 1981). This could not be demonstrated in the present study. Ultrasonographic abnormalities should 279 therefore be considered of only limited value in the decision-making process for obtaining a PUC. 280 However, an increased wall thickness and/or irregular luminal surface may especially warrant 281 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.

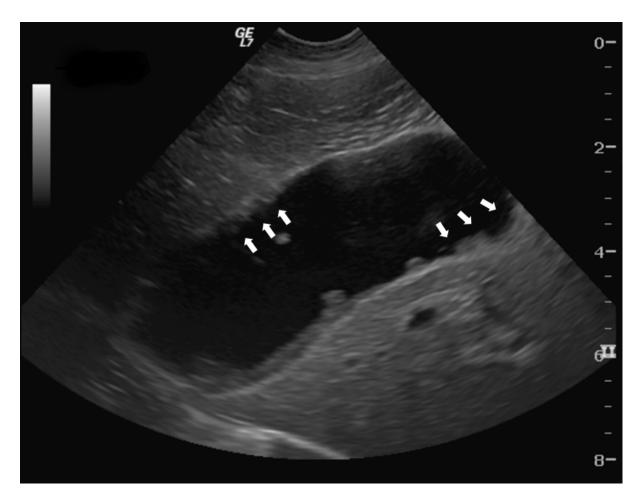
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304 No conflicts of interest have been declared.

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

# 359

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	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	АКІ	Died	Increased wall thickness (mean 1.2mm)	

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

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 immunemediated thrombocytopenia, SIRS systemic inflammatory response syndrome, WHWT West Highland White Terrier

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# Table 2Results of multilevel, multivariable analyses for associations with positive bile culture following 300<br/>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; Cl, confidence interval; OR, odds ratio;

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