

Percutaneous Ultrasound-guided Cholecystocentesis and Bile Analysis for the Detection of *Platynosomum* spp.-Induced Cholangitis in Cats

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Background: Examination of bile could be useful to diagnose *Platynosomum* spp.-induced cholangitis in cats. Obtaining bile via percutaneous ultrasound-guided cholecystocentesis (PUC) is possible but raises safety concerns in cats with severe cholecystitis.

Objectives: The objectives of this study were to investigate the use of PUC to collect bile samples from cats with known platynosomosis and to determine if bile analysis could be a diagnostic test.

Animals: Twenty-seven free-roaming cats positive for *Platynosomum* spp. eggs via fecal examination.

Methods: In this prospective study, fecal egg counts were performed by double centrifugation with Sheather's solution. Bile was collected using PUC from anesthetized cats. Egg counts in bile were performed with a stereoscope. Euthanasia and postmortem examination were performed immediately after PUC.

Results: All cats had ultrasound (US) evidence of cholangitis or cholecystitis. Thirty-nine PUCs were performed with 14 cats having 2 PUCs 12 or 24 days apart. Postmortem examinations showed no overt gallbladder damage or leakage but fresh blood was noted in the gallbladder lumen of 3 cats. Median *Platynosomum* spp. egg counts were higher in bile (1450 eggs/mL; IQR, 400; 5138 eggs/mL) as compared to feces (46 eggs/mL; IQR, 10; 107 eggs/mL) ($P < .001$).

Conclusion and Clinical Importance: Bile egg count analysis is an alternative method with higher egg counts as compared to fecal egg count analysis for the diagnosis of platynosomosis. Obtaining bile via US guidance is technically feasible and safe in cats with cholangitis/cholecystitis. Cholecystocentesis and bile analysis are especially relevant for those cats with chronic cholangitis/cholecystitis and negative fecal egg counts for *Platynosomum*.

Key words: Cholecystitis; Chronic cholangitis; Feline; Lizard poisoning; Platynosomiasis.

The World Small Animal Veterinary Association (WSAVA) working group on hepatobiliary diseases¹ defines feline inflammatory liver diseases of cats as 3 distinct histopathologic groups: neutrophilic (acute and chronic), lymphocytic, and chronic cholangitis caused by liver flukes such as *Platynosomum* spp. and *Amphimerus pseudofelineus*.²

As a taxonomic controversy exists regarding the *Platynosomum* species names, they are often collectively referred to as *Platynosomum* spp.³ This parasitic disease is also known as “lizard poisoning” as it is assumed that most affected cats acquire the parasite by eating infected lizards found in the tropics and subtropics.³ The fluke resides in the gallbladder and biliary ducts of the infected cat⁴ creating inflammation within bile ducts and portal areas.

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Abbreviations:

FCP	Feral Cat Project
US	ultrasound
PUC	percutaneous ultrasound-guided cholecystocentesis
RUSVM	Ross University School of Veterinary Medicine

Prevalence of platynosomosis is high in endemic areas such as the Caribbean island of St. Kitts, where 81% of feral cats have positive fecal results.⁵ In endemic regions, many infected cats have both high fluke burdens and chronic infections, which play a role in pathogenesis.^{6,7}

Nonspecific signs of acute infection include inappetence and lethargy. With high fluke numbers, chronic mucoid diarrhea, and icterus can occur and persist until the cat dies.^{4,8} In experimental infections, abdominal pain, icterus, and hepatomegaly occur between the 7th and 16th week,^{9,10} but such signs were transitory in nature. Nonspecific clinical signs can reoccur once the disease becomes chronic as the adult flukes persist for what is considered a long period of time.

In cats from endemic areas, the antemortem diagnosis largely relies on a combination of clinical and ultrasonographic findings, with confirmation made by detecting eggs in the feces.³ Factors which influence the accuracy of fecal diagnosis of *Platynosomum* spp. infections include the fecal analysis method, the intermittent shedding of eggs, and the low number of eggs per gram of feces.¹¹

Percutaneous ultrasound-guided cholecystocentesis (PUC) has been described therapeutically¹² and used for collecting bile for diagnostic purposes in animals.^{5,13–17} Bile is considered the sample of choice for

culture in the diagnosis of cholangitis and cholecystitis in the cat, where ascending bacterial infection is the most likely etiology.¹⁸ To our knowledge, bile has not been used to diagnose fluke-induced chronic cholangitis.

Percutaneous ultrasound-guided cholecystocentesis is considered a safe, minimally invasive, and technically simple procedure in healthy cats.¹⁴ However, concerns have been raised about its safety in cats with cholangitis and specifically when there is disease of the gallbladder.¹⁹ To avoid bile leakage into the peritoneum, complete emptying of the gallbladder of its content¹⁴ and a transhepatic approach to access the gallbladder through its hepatic attachment are recommended.

Ultrasonographic changes of cholangitis include gallbladder distention, hyperechoic gallbladder walls, tortuous bile ducts, enlarged, irregularly shaped liver, heterogeneous and hyperechoic parenchyma.^{20,21} Unfortunately, these changes are not specific for fluke-induced chronic cholangitis as neutrophilic and lymphocytic cholangitis can result in similar ultrasonographic changes.²¹

This prospective observational study examined the clinical usefulness and complications associated with using PUC and bile analysis to diagnose *Platynosomum* spp. infection in cats living on St. Kitts. In addition, it describes the ultrasonographic and histologic findings of cats with platynosomiasis.

Materials and Methods

Study population

Cats were recruited from the RUSVM Feral Cat Program (FCP), a trap, spay/neuter, and release program during the period August 2014 to July 2015. All cats in the FCP are tested for FIV using a commercially available patient-side test^a, and cats positive for FIV are euthanized as part of a test and remove policy. All cats used in this study were FIV-positive and designated for euthanasia. Twelve cats were recruited directly from the FCP and all except 1, which was rehomed, were euthanized after the ultrasound (US). Fifteen cats (FIV-positive) were allocated from FCP to another study assessing the efficacy of praziquantel treatment of platynosomiasis. These cats had US and PUC performed twice, once before praziquantel treatment and a second time either 12 days (7 cats) or 24 days (8 cats) later.

All US and PUC procedures were performed under a short duration anesthesia, using a combination of ketamine hydrochloride (3–5 mg/kg),^b buprenorphine hydrochloride (0.01–0.016 mg/kg),^c and dexmedetomidine hydrochloride (11–20 µg/kg)^c administered intramuscularly. The cats were euthanized by the intravenous administration of pentobarbital while they were under general anesthesia and full gross postmortem examinations were conducted. Food was withheld for 12 hours before anesthesia for each procedure, but water was available *ad libitum*. The cats housed for the praziquantel dosing study were observed daily for appetite, habitus, urination, and defecation. All procedures in the PUC study as well as those within the FCP and the praziquantel study were conducted under RUSVM's Institutional Animal Care and Use Committee (IACUC)-approved protocols. The cat housing facility also was inspected and approved by the IACUC.

Ultrasound and PUC

During the anesthesia, which was at the time of FIV screening in all cats, the ventral abdomen was clipped by using a clipping

machine,^d and abdominal ultrasonography was performed using a multifrequency [8 to 14 MHz] sector scanner.^e Liver and biliary tract findings were recorded.²² Gallbladder and bile duct wall measurements were made on the near wall when oriented perpendicular to the sound beam to improve accuracy.²³ Percutaneous ultrasound-guided cholecystocentesis was conducted, with a 22-gauge, 1.5-in (3.81-cm) needle and 5-mL syringe, via a right-sided ventral abdominal, transhepatic approach after wiping the ventral abdominal skin with ethanol as an antiseptic. This was achieved by allowing at least 2 cm of hepatic parenchyma positioned between the gallbladder wall and the subcutaneous fat and attempting to completely empty the gallbladder of its content. Large vascular structures were avoided with the aid of color flow Doppler. The PUC attempt was abandoned if the gallbladder was located in a position not easily accessible to needle centesis because of the degree of bile fill.

The volume of bile collected by a single attempt at PUC was recorded and the remaining bile volume in the gallbladder was estimated by US. Organoleptic examination of bile included color and turbidity. If 3 mL or more was collected, 2 mL was submitted to the RUSVM Diagnostic Laboratory for aerobic and anaerobic cultures. The hepatobiliary system was examined again at the end of the abdominal US procedure to assess potential damage or bile leakage as determined by visualizing hemorrhage or hematoma formation²³ or loss of continuity of the gallbladder wall.²⁴

Fecal and bile analyses

Feces were collected from the litter tray and also during the postmortem examination. Two grams of feces was analyzed by double centrifugation with Sheather's sugar flotation solution, the preferred fecal analysis method for finding *Platynosomum* eggs.²⁵ All eggs collected on the coverslip were counted and divided by the grams of collected feces.

Bile collected on each PUC sample was subject to direct microscopic examination; two 10-µL or 20-µL samples of bile were placed on a slide or Petri dish, and eggs were counted with a stereoscope at 50× to 100× magnification. Counts were averaged to calculate eggs per milliliter. The total bile egg count was calculated as the product of the bile aspirate volume and egg count per milliliter.

Histopathology

Euthanasia and postmortem examination were performed immediately after US and PUC. The liver, gallbladder, peritoneum, and juxtaposed organs were examined in situ to determine if there was evidence of bile leakage or hemorrhage; the PUC puncture site was also examined for evidence of leakage or lacerations. Sections of gallbladder, common bile duct, and the tips of each liver lobe were collected and preserved in 10% neutral buffered formalin and later trimmed, routinely processed, and embedded in paraffin, cut at 5 microns and stained with hematoxylin and eosin for microscopic assessment by a board-certified pathologist.

Statistical analysis

Excel software was used to calculate the median and interquartile range (IQR) of the continuous data. Statistical analyses were performed by a statistical software package.^f Distribution of continuous data was verified with a Kolmogorov-Smirnov test and differences between bile and fecal egg counts were compared using a Wilcoxon signed-rank test. Significance was set at a *P* value of ≤0.05.

Results

Twenty-seven free-roaming feral cats were included in this study. Twenty-three were intact males and 4 were intact females. All were positive for FIV antibodies.

Ultrasonography

Nineteen of the 27 cats had hepatic changes on US consisting of hyperechogenicity (16/27), heterogeneity (13/27), enlargement (6/27), and/or irregular margins (3/27). The remaining 8 cats had no detectable changes related to the liver. Portal and hepatic vessels were tapering in all cats.

Twelve cats had no changes in the gallbladder. The gallbladder was appropriately distended for the duration that food was withheld in 10 cats (37%). The wall was >2 mm thick and hyperechoic in 25.9% of cats ($n = 7$) (Fig 1). Gallbladder content was echogenic in 10 cats and anechoic in the remainder. Gallbladder shape varied from pyriform (15/27), to oval (9/27) to round (3/27). Maximum diameter was measured in 23 cats and the median diameter was 1.6 cm (IQR: 1.3 cm; 1.95 cm).

The common bile duct diameter in 27 cats had a median of 3.9 mm (IQR: 2.85 mm; 5.25 mm), but 12 cats had a diameter exceeding 4 mm (Fig 2). Periductal hyperechogenicity was visible in 13 of 27 cats (Fig 3). The course of the common bile duct was tortuous in 16 cats ($n = 16$). One cat had evidence of intrahepatic biliary dilatation.

PUC

On only 2 occasions (small-sized cat; lactating female) was it deemed technically too challenging to attempt the PUC. In general, PUC was easily performed with a 100% yield of bile aspiration in 39 procedures. The median volume of bile collected was 3 mL (IQR:



Fig 1. A longitudinal image of the liver (head to the left) with the cat in dorsal recumbency and imaged from the right cranio-ventral abdomen (8 MHz). The image demonstrates a distended gallbladder that is oval in shape with thickened and hyperechoic walls.



Fig 2. A longitudinal image of the liver (head to the left) with the cat in dorsal recumbency and imaged from the right cranio-ventral abdomen (8 MHz). This image demonstrates distended common bile duct and hyperechoic thickened common bile duct wall.

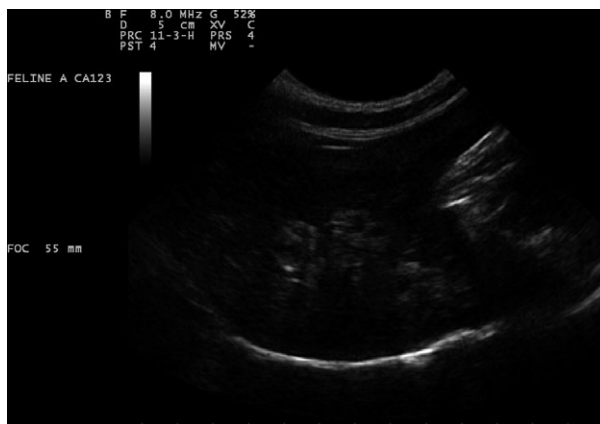


Fig 3. A longitudinal image of the liver with the cat positioned in dorsal recumbency. There are multifocal hyperechoic lesions throughout the parenchyma with central anechoic fluid. This represents an image that bisects the bile ducts in cross section and periductal hyperechogenicity.

1.95 mL; 5.8 mL). The gallbladder could not be emptied completely in 27/39 PUCs as judged via US.

Bile analysis and culture results

Twenty-eight of 39 samples were turbid. Color was dark green in 15 samples and lighter shades of green in the remaining 24 samples. Eighteen samples were cultured from 13 cats; 5 cats were cultured twice: once on initial PUC and again on a second PUC. Culture results (positive or no growth) were consistent between the 2 samples. *Escherichia coli* organisms were cultured from bile of 6 cats, of which 3 cats were cultured positive on 2 occasions.

Egg counts

Of the 27 cats recruited, fecal and bile egg counts were obtained on 24 and 23 cats respectively. Fecal counts were not performed if feces could not be

obtained. Bile egg counts were not performed on 1 cat that did not undergo a PUC because of difficulty experienced in approaching the gallbladder as a result of her small body size. For the remaining 3 cats, bile was collected via PUC and the presence of *Platynosomum* spp. eggs was confirmed but not quantified.

Egg counts were nonparametric in their distribution ($P < .001$). The median and 25th and 75th IQR of the egg count for *Platynosomum* spp. on bile and feces was 1450 eggs/mL (400; 5138 eggs/mL) and 46 eggs/g (10; 107 eggs/g), respectively. There was a significantly higher egg count (when adjusted to mL and g equivalents) in direct examination of bile collected by PUC compared to feces analyzed by double centrifugation ($P < .001$).

Complications

No evidence of complications (bleeding from the liver, bile leakage from the gallbladder, or accumulation of free peritoneal fluid) were found at the time of completion of the first US (27 cats) nor at the time of the second follow-up US (15 cats). *In situ* examination of the hepatobiliary system did not detect hemorrhage or bile peritonitis. At gross postmortem examination, a very small puncture site was visible in the gallbladder wall, presumably because of the PUC, in all cats. In 3 cats, the bile was described as blood contaminated at postmortem, but no bleeding was noted in the liver or into the peritoneal cavity.

Histopathology

Histologic evidence of cholangitis and pericholangitis was observed in 25 of the cats examined. Microscopic findings ranged from a mild lympho-plasmacytic pericholangitis (5/25) to a severe fibrosing cholangitis and pericholangitis, cholangiectasis, and occasional adenomatous hyperplasia of the bile duct epithelium (4/25 cats) (Fig 4). In severely affected cats, inflammatory

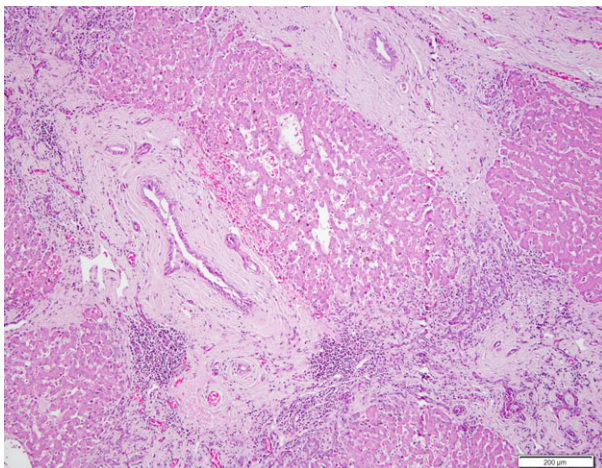


Fig 4. Severe chronic pericholangitis with interface hepatitis and bridging fibrosis, 10X, HE.

changes within portal triads extended into the adjacent hepatic parenchyma leading to a periportal interface hepatitis characterized by hepatocellular loss, bridging portal fibrosis, and occasional bile duct proliferation (Figs 5 and 6). A few metazoan parasites, identified as *Platynosomum* spp., were present within the lumen of moderately to markedly distended bile ducts in 4 cats (Fig 7). Hyperplastic adenomatous cholecystitis was detected in 23/25 cats and characterized as mild (9) or moderate (14). The inflammatory cell infiltrate associated with liver and gall bladder lesions was primarily composed of lymphocytes and plasma cells but a significant number of eosinophils were occasionally observed. The associations between severity of cholangitis and cholecystitis, and hepatic and biliary tract ultrasonographic changes are summarized in Table 1.

Discussion

This study demonstrated the diagnostic utility and safety of PUC performed in a group of cats diagnosed

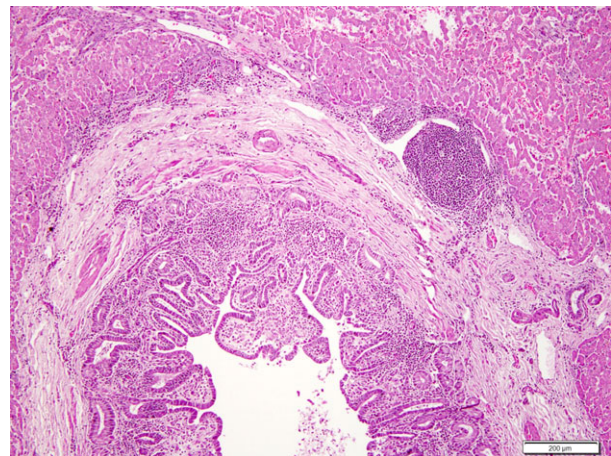


Fig 5. Chronic cholangitis with cholangiectasis and prominent adenomatous hyperplasia of the bile duct epithelium, 10X, HE.

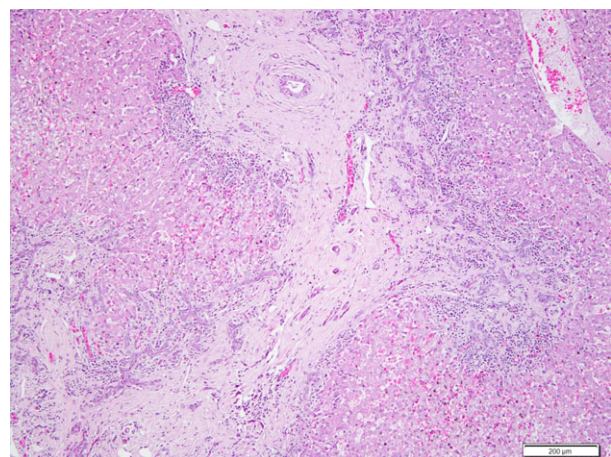


Fig 6. Chronic fibrosing pericholangitis with marked proliferation of bile ducts, 10X, HE.

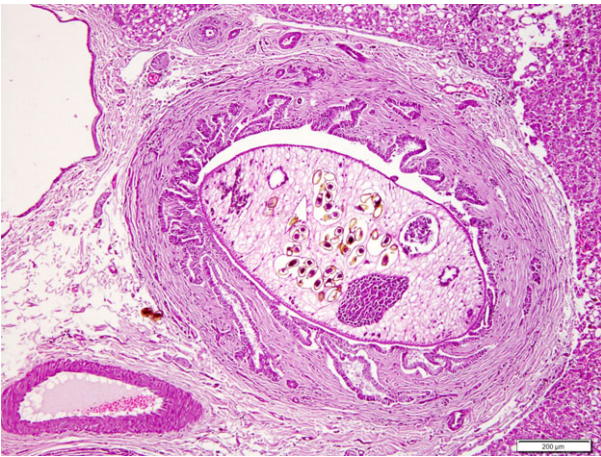


Fig 7. A mature fluke almost completely obliterates the lumen of a dilated thick-walled bile duct, 10X, HE.

with *Platynosomum*-induced chronic cholangitis and accompanying cholecystitis. It demonstrated that bile, obtained via PUC, can be used to diagnose platynosomosis. Bile egg counts were found to be more sensitive in diagnosing platynosomosis than fecal egg counts. In addition, 10- to 20- μ L drops of bile can be examined in-house easily and rapidly for *Platynosomum* eggs.

In a previous US investigation of *Platynosomum* spp., Salamao et al. (2005) found a higher number of female cats infected, with the proposed explanation being the higher risk of exposure when female cats teach their young to hunt.²² Because our study used only free-roaming, FIV-positive, feral cats, males were overrepresented. The epidemiology of FIV indicates that it is predominantly transmitted by fighting males.²⁶

Ultrasound is considered a fairly accurate diagnostic test for cholangitis in cats, with sensitivity and specificity reported at 87% and 90%, respectively.²⁷ Hepatomegaly and a heterogeneous pattern, created by areas of hyperechoic parenchyma surrounding the portal regions, were changes of cholangitis observed in our study and others.²¹ Cats with histologically confirmed lymphocytic and chronic cholangitis have liver texture described as coarse or nodular and loss of portal vein clarity.²⁸ A more recent study found cats with lymphocytic cholangitis had hepatomegaly (21%), microhepat-

ica (7%), and hyperechoic (36%) or hypoechoic (14%) echogenicity.²¹

Ultrasonographic evidence of extrahepatic biliary obstruction was found in 16 cats based on distension of the gallbladder (10/27) and/or common bile duct (6/27). Gallbladder distension is considered the earliest ultrasonographic change of extrahepatic biliary obstruction experimentally in dogs,²⁹ although the lack of this finding does not exclude the possibility of obstruction in the cat.³⁰ In 12 cats of this study, the common bile duct exceeded 4 mm, which is considered the upper limit of normal in a cat and in 6 of those cats, it exceeded 5 mm, which suggested extrahepatic biliary obstruction.³⁰ However, the common bile duct in the cat can remain distended after the obstruction is relieved because of inflammation and prolonged distention reducing the elasticity of the duct. One cat had histopathologic evidence of intrahepatic cholestasis, which is always a sequela to extrahepatic biliary obstruction. In dogs, intrahepatic cholestasis develops at least 5- to 7-day postobstruction.²⁹

Gallbladder walls were hyperechoic and thicker than 2 mm in 7 cats. Normal gallbladder wall is undetectable ultrasonographically, but measurements reported in visible gallbladder walls were 0.4–0.9 mm, with gallbladder wall ≥ 1 mm accurate in predicting gallbladder disease in cats.³¹ All cats in this study had a gallbladder wall thickness that exceeded this reference limit.

In another study where ultrasonographic changes were compared to histopathology, acute cholecystitis was associated with double-rimmed, thickened, gallbladder walls, whereas thinner, single layered, hyperechoic walls were more characteristic of chronic or mild inflammation.³¹ The gallbladder walls could be defined as hyperechoic and thickened in the majority of cats in our study, which would be characteristic of chronic cholecystitis.

The frequency of gallbladder sludge (37%) was higher in our study than reported in a hospital population of cats presented for abdominal US (14%).³² While prolonged fasting because of anorexia has been proposed to explain gallbladder sludge³², a number of reports claim that gallbladder sludge in cats, unlike the dog but similar to humans, is a significant finding on US examination. All the cats in this study were starved 12 hour before general anesthesia, but none of the cats

Table 1. The severity of cholangitis and cholecystitis determined by histopathology and the corresponding ultrasound findings in 25 cats that underwent abdominal ultrasonography and subsequent euthanasia with histopathology of the liver and biliary tract

	N	Cholecystitis	Hyperechoic	Heterogenous	Dilated/tortuous CBD
Mild cholangitis	5	None: 1 Mild: 3 Moderate: 1	2	1	1
Moderate cholangitis	13	None: 1 Mild: 6 Moderate: 6	8	6	11
Severe cholangitis	6	Moderate: 6	4	4	6

N, number of animals; CBD, common bile duct.

had anorexia recorded during their hospitalization. Gallbladder sludge has been shown to be associated with cats diagnosed with hepatobiliary or gastrointestinal disease (20%),³¹ and extrahepatic biliary obstruction (62%)³³, therefore cholestasis appears to be a significant contributor.

The volume of bile collected (3 mL; IQR: 1.95 mL; 5.8 mL) was considered sufficient to perform diagnostic testing, including bile egg count, and aerobic and anaerobic cultures. In healthy cats, the mean volume of bile collected by PUC has been reported to be 1.8 ± 0.8 mL (range 0.9–3 mL).¹⁴ In a report describing acute cholecystitis in 3 cats, the volume of bile collected by PUC was 1–2 mL.¹⁹

A 22-gauge needle can be an insufficient gauge should the bile be thick or inspissated.²⁰ This was not the case in any of the cats sampled in our study, in which the bile was found to be liquid. However, turbidity was noted in 28 samples as well as flocculent material, most likely inflammatory debris. *Escherichia coli* infection was identified in 46% of the cats in which bile was submitted (13 cats) for bacteriologic culture. Bile in healthy cats is sterile.¹⁴ *Enterobacteriaceae* organisms are postulated to originate from the proximal duodenum, and inoculated by retrograde infection of the biliary tree by migrating flukes. This investigation supports the recommendation of using gram negative spectrum, bile-excreted antibiotic treatment in fluke-infected cats.²⁰

When fecal and bile egg counts were compared, bile egg counts were significantly more sensitive. Thus, bile analysis would be an appropriate test to perform in cats with negative fecal egg counts but with serum biochemistry and ultrasonographic findings suggestive of cholangitis to rule out platynosomosis. The additional advantage of this technique is that only 1 or 2 drops of bile without the need for centrifugation are all that is required for direct microscopic confirmation of *Platynosomum* flukes.

The PUC was successfully performed on every attempt although the gallbladder was rarely completely emptied. Draining the gallbladder is advisable to prevent leakage through the needle centesis lesion.²⁰ No apparent complications were noted in our study despite incomplete emptying in 69% of the procedures performed. Ultrasonographic evidence of severe cholecystitis may be a possible contraindication to PUC in which case surgical exploration may be considered.¹⁹ Furthermore, the presence of emphysematous cystitis caused by anaerobic infections, diagnosed by the presence of intraluminal gas, is a contraindication for PUC, because of the risk of sepsis.³⁴

In a previous study examining the use of PUC in healthy cats, abdominal hemorrhage was encountered after the first procedure using this technique, thereafter a direct gallbladder approach was conducted without any complications.²² In the same study, 4 cats demonstrated transient inappetence, 4 had mild, transient (2 days) abdominal pain, but there were no gross or histologic lesions in abdominal organs at postmortem.¹⁴ Percutaneous ultrasound-guided cholecystocentesis was described in 3 cats allowing a noninvasive diagnosis of

neutrophilic cholecystitis and acute neutrophilic cholangitis.¹⁹ One of these cats suffered gallbladder rupture and bile peritonitis. None of the cats in our study had evidence of acute or severe cholecystitis consistent with necrosis, ie, trilamination of the gallbladder wall²⁰ or emphysematous cystitis,³⁴ although cholecystitis was suspected in all the cats that were sampled and the procedure was well tolerated.

Microscopic findings within the liver and gallbladder of cats with platynosomosis were similar to those reported in the literature^{3,35,36} but there was no apparent ability to predict severity of pathology based on the ultrasonographic changes. Selective and restrictive sampling for histologic assessment as in our study may have underrepresented the true severity of the hepatic pathology as lesions are multifocal and can be missed during the sampling procedure.³⁷

There are 4 limitations of this study. Not all bile samples were submitted for culture and it is difficult to make inferences about bacterial infections in chronic cholangitis because of the small sample size. Secondly, the histopathology is not representative of the entire liver as only small areas were submitted for histopathology (the majority of the liver was processed for fluke counting). This misrepresents the severity of liver pathology as multifocal lesions were expected.³⁷ Third, all of the cats had natural *Platynosomum* spp. infections of unknown fluke maturity and chronicity and intensity of the infection. Lastly, all cats included in this study were FIV-positive which could account for higher fluke burdens, and be more susceptible to concurrent bacterial cholangitis. The results of this study do not necessarily apply to FIV-negative cats. Future studies should examine bile cytology and examine the relationship between it, histopathologic, and ultrasonographic changes and severity and chronicity of the fluke infection.

Footnotes

^a SNAP FIV/FeLV Combo Test, IDEXX Laboratories, Westbrook, ME, USA

^b Pfizer Inc, New York, NY

^c Dexdomitor manufactured by Orion Pharma, Finland and distributed by Zoetis Inc, Kalamazoo, MI

^d Oster Clipper, McMinnville, TN, USA

^e Esoate, MyLab™, Genova, Italy

^f SPSS statistics 22, IBM, New York, NY

Acknowledgments

This study was conducted at Ross University School of Veterinary Medicine (RUSVM). Approval for recruitment of animals from the Feral Cat Project (FCP) for US, PUC, fecal and bile analysis, and post-mortem organ collection, was under the Institutional Animal Care and Use Committee (IACUC), RUSVM Protocol 14-3-009; housing and euthanasia were

approved by IACUC, RUSVM Protocol 15-1-004. Partial funding for this study was provided under a grant from the National Center for Veterinary Parasitology at Oklahoma State University. Some data from this study were presented in a poster abstract at the World Association of Veterinary Laboratory Diagnosticians (WAVLD) Symposium on the 15th June 2015.

Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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