
This is the author’s final accepted version.

There may be differences between this version and the published version. You are advised to consult the publisher’s version if you wish to cite from it.

http://eprints.gla.ac.uk/135855/

Deposited on: 11 April 2017

Enlighten – Research publications by members of the University of Glasgow
http://eprints.gla.ac.uk
The Diagnosis of Pancreatic Disease in Feline Platynosomosis

Liza S Köster1*, Linda Shell1, Jennifer Ketzis2, Sreekumari Rajeev2, and Oscar Illanes2

1Department of Clinical Sciences, Ross University School of Veterinary Medicine, Basseterre, West Indies

2Department of Biomedical Sciences, Ross University School of Veterinary Medicine, Basseterre, West Indies

Running head: feline pancreatic disease and Platynosomum spp. infection.

Keywords: cat, cobalamin, fPL, pancreatitis, platynosomiasis, platynosomosis, Platynosomum spp., fTLI

Abbreviations:

EPI: exocrine pancreatic insufficiency

fPL: feline pancreatic lipase

fTLI: feline trypsin-like immunoreactivity

IQR: interquartile range

RI: reference intervals

*Corresponding author: Liza S Köster, BVSc, DECVIM-CA, School of Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Bearsden Road, Glasgow, G61 1QH, UK, email: liza.koster@glasgow.ac.uk
Abstract

Background Platynosomum spp. are cat-specific parasitic trematodes that parasitize the biliary ducts and gallbladder. Due to the common connection to the major duodenal papilla of the pancreas and common bile ducts in addition to the periductal proximity of the pancreas, it is possible that platynosomosis could cause pancreatitis.

Hypothesis/Objectives To determine if platynosomosis, a commonly diagnosed parasitic disease in cats on St. Kitts, has any association with pancreatic disease. To investigate this possibility, the pancreas of free roaming cats with naturally acquired platynosomosis were evaluated via ultrasound, serum concentrations of fPL, cobalamin, folate and fTLI, and histopathology.

Animals: Twenty free roaming, young adult feral cats, positive for feline immunodeficiency virus, and diagnosed with Platynosomum spp. infection via fecal analysis.

Methods: The liver, biliary system, and pancreas were evaluated via ultrasonography during a short duration anesthesia. Serum concentrations of fPL, fTLI, folate, and cobalamin were measured. Sections of the right limb, left limb, and body of the pancreas were evaluated histopathologically using H&E stain.

Results: None of the cats had sufficient criteria to fulfill the ultrasonographic diagnosis of pancreatitis. One cat had an elevated fPL concentration in the range consistent with pancreatitis. Four cats had cobalamin deficiencies and eleven had abnormal folate concentration. The fTLI concentration was equivocal for the diagnosis of exocrine pancreatic insufficiency in one cat. With a single exception, histopathology changes, when present (n=12), were mild, non-specific and predominantly characterized by lymphocytic infiltrates and fibrosis. The exception was a cat which presented a chronic interstitial and eosinophilic pancreatitis of slightly increased severity likely the result of platynosomosis.
Conclusion and clinical importance: The results of this study suggest that platynosomosis rarely induce pancreatic damage in cats. With only one exception, chronic pancreatitis diagnosed in cats with fluke-induced cholangitis and cholangiohepatitis was subtle and interpreted as an incidental background lesion unrelated to platynosomosis.
Introduction

Platynosomum spp. are cat-specific parasitic liver trematodes (flukes) that occupy the hepatic ducts and gallbladder (1) of cats from tropical and subtropical regions of the world. (2) Gross pathology findings in Platynosomum spp. infected domestic cats have included hepatomegaly, a yellow and friable liver, biliary duct distension, increased bile consistency with visible flukes, mesenteric lymphadenopathy, and ascites. (1) Both immature and mature flukes of the Platynosomum spp. have been found in the pancreatic duct of cats. (3) Due to the common connection to the major duodenal papilla of the pancreatic and common bile ducts, in addition to the periductal proximity of the pancreas, it is possible that a liver fluke infection could cause pancreatitis. Pancreatitis has been reported in a cat infected with Eurytrema procyonis, the Raccoon pancreatic fluke. (4)

Hepatobiliary changes on ultrasonography in domestic cats infected with Platynosomum spp. are not specific to the fluke infection but 29% of cats have associated bile duct or gallbladder alterations. (5) Abnormalities included distention of the gallbladder, with hyperechoic walls and anechoic bile content and tortuous and distended bile ducts. Further, livers in infected cats are described as enlarged, irregular in shape, and hyperechoic with heterogeneous texture. Ultrasonographic descriptions of the pancreas in cats with platynosomosis have not been described.

In the majority of feline pancreatitis cases, the etiology is unknown. Pancreatitis in cats has been classified as acute, chronic or chronic-active. Another classification scheme is based on histopathologic findings: necrotizing pancreatitis, pancreatic fibrosis with inflammation or without inflammation. (6) A histologically distinct suppurative pancreatitis, which is an acute form of the disease, has been documented in cats. (7) Acute pancreatitis in cats, which is usually
a clinically apparent condition, can occur in any age of cat, obese or underweight.(7). Although
the Siamese breed was overrepresented in one study (7) a review of other published cases could
not support that finding. Documented conditions or infectious diseases associated with
pancreatic pathology in cats are numerous and include: Toxoplasmosis(8, 9), flukes (Eurytrema
procyonis)(4), trauma(10), hypcobalaminemia(11), triaditis(12), hepatic lipidosis(13), diabetes
mellitus(14), and cavity effusions.(15) It is believed that acute and chronic pancreatitis occurs
commonly in cats. In one study of cats that were clinically normal at the time of death, the
prevalence of pancreatitis was 45% based on histopathology of pancreas, the prevalence of
pancreatitis increased to 67% when cats that were euthanized for a specific disease including
both gastrointestinal and non-gastrointestinal conditions.(16) Unfortunately ante-mortem
diagnosis of pancreatitis in domestic cats remains challenging due to inconsistent clinical
chemistries and the lack of specific clinical signs. Many cats suffer from subclinical pancreatitis
which can impair successful management of the comorbid diseases associated with pancreatitis.
One third of cats with diabetes mellitus are estimated to have subclinical pancreatitis based on
either elevated fPL or abdominal ultrasonography changes, which could have implications on
achieving remission.(14) In addition, the magnitude of fPL concentration has shown to be
associated with outcome in acute pancreatitis and is considered a prognostic variable in critically
ill cats.(17) The unfavorable reputation of abdominal ultrasonography in its ability to discern
pancreatic pathology has been recently highlighted in several studies where the correct diagnosis
of pancreatitis was made in a paltry 20% to 54% of cases.(18-20) While pancreatic
ultrasonography has the advantage of being a non-invasive method of assessing morphology, it is
limited by the lack of specificity and dependent on the skills of the sonographer. However,
ultrasonography is considered to be a reasonable clinically diagnostic tool when used in
combination with serological markers of pancreatitis. The current test of choice for non-invasive
diagnosis of pancreatitis is the assessment of feline pancreatic specific lipase (fPL)
concentration. (21) Recently a study demonstrated that the sensitivity and specificity of
ultrasonography in detecting acute pancreatitis diagnosed by abnormal fPL, was 84% (95%
confidence interval = 60–97%), and 75% (95% confidence interval = 48–93%) respectively. (13)
This same study found that ultrasonographic changes of pancreatic limb thickening, loss of
margination and peripancreatic fat hyperechogenicity in combination with an abnormal fPL
assay were highly indicative of acute pancreatitis in cats. Computed tomography offers little
additional benefit in the ability to diagnose pancreatitis in cats, with a sensitivity of 20% reported
in one study. (18) Endosonography is reported to have superior visualization of the normal
pancreas with better resolution in pancreatic pathology, however the diagnosis of pancreatitis
would not have been altered in the study that compared its usefulness to that of abdominal
ultrasonography, making the clinical application of this invasive diagnostic test limited. (22)

Exocrine pancreatic insufficiency (EPI) is assumed to develop as a consequence of end-
stage chronic pancreatitis in the feline species; however cats less than six months of age also can
develop EPI. (23) Reported prevalence ranging from 0.013 – 0.103% has been increasing,
possibly due to increased awareness and availability of commercial assays. (23-26) Typically cats
diagnosed with EPI have hypocobalaminemia due to malabsorption, while folate concentrations
are normal or increased. (25, 26) The gold standard for diagnosis of EPI in domestic cats has not
been established, but feline trypsin-like immunoreactivity (fTLI) has been validated for use in
serum from starved cats, with concentrations ≤8 ug/L consistent with the diagnosis of EPI. (27)
While very few of the cats have had biopsy confirmation of the disease the diagnosis is
supported by clinical response to pancreatic enzyme supplementation, reported in 87% of
cases. While one study diagnosed EPI using fTLI with a cut-off of 12 µg/L, the recommendation of Gastrointestinal Laboratory, Texas A&M, refers to a cut-off of 8 ug/L for the diagnosis and between 8-12 µg/L as equivocal.

The objective of this study was to determine if platynosomosis, a commonly diagnosed parasitic disease in cats on St. Kitts, could be associated with pancreatitis and its sequela EPI. To investigate this, the morphology, functional reserve, and architecture of the pancreas of free roaming cats naturally infected with *Platynosomum* spp. were evaluated using abdominal ultrasound, serum concentrations of fPL, fTLI and cobalamin, and histopathology.

**Materials and Methods**

This prospective study was conducted in conjunction with a larger investigation on the diagnosis and treatment of platynosomosis in domestic cats on St. Kitts, West Indies. Cats were recruited from the Ross University School of Veterinary Medicine (RUSVM) Feral Cat Program (FCP), a trap, spay/neuter and release (TNR) program, during the period August 2014 to July 2015. All procedures in this study as well as those within the FCP and the larger platynosomosis study were conducted under the following RUSVM Institutional Animal Care and Use Committee (IACUC), approved protocols: FCP (13-9-017), FCP retrovirus testing (15-2-006), necropsy and organ harvesting (14-3-009), and *Platynosomum* spp. investigation (15-1-004). The cat housing facility also was inspected and approved by the IACUC. As per the Feline Immunodeficiency Virus (FIV) “test and remove policy” of the TNR program, all cats more than six months old were tested using a patient side commercial FIV antibody test and euthanized if tested positive. Only FIV positive cats were included in the study. A fresh stool sample was collected for diagnosis of *Platynosomum* spp. infection by standard parasitological methods described elsewhere.
Prior to euthanasia, eight cats were allocated to another study which investigated the efficacy of praziquantel as treatment of platynosomosis. These eight cats were examined and euthanized at either 12 days (n=4) or 24 days (n=4) post FIV testing and after treatment with praziquantel. Their appetite, appearance, urination and defecation habits were monitored twice daily.

Food was withheld for 12 hours prior to anesthesia for each procedure, but water was available *ad libitum*. Abdominal ultrasonography was performed under a short duration anesthesia, using a combination of ketamine hydrochloride (3-5 mg/kg), buprenorphine hydrochloride (0.01-0.016 mg/kg), and dexmedetomidine hydrochloride (11-20 µg/kg) administered intramuscularly. All cats were euthanized using pentobarbital (1ml/4.5kg) administered intravenously while they were anesthetized.

Ultrasonography was performed by an internal medicine specialist, using an 8.5 MHz to 14 MHz sector scanner. All images were saved onto the server (DICOM). The cat was positioned in dorsal recumbency and the abdomen was clipped using electronic clippers. After a complete abdominal ultrasound, the pancreas was evaluated and measured using electronic calipers in either longitudinal or transverse plane, whichever was most appropriate for the area being examined.

The procedure for pancreatic measurements and grading followed the criteria recommended by Williams *et al.* (2013). Thickness was the maximum ventro-dorsal width measured using electronic calipers, with greater than 1 cm considered abnormal. As per the classification scheme developed by Zimmerman *et al.* (2013), four ultrasonographic criteria of pancreatitis (change in echogenicity, hyperechoic peripancreatic fat, pancreatic enlargement, and peritoneal fluid) were considered. Finding three or more of these criteria was
Serum, collected at the time of euthanasia, was stored at -80°C until it was shipped to IDEXX laboratory for determinations of fPL, folate, cobalamin and fTLI. Reference intervals (RI) for fPL was reported as 0 – 3.5 µg/L. In addition, as part of the gastroenterology panel offered by IDEXX laboratories, folate (RI: 8.9 – 19.9 µg/L), cobalamin (RI: 276 – 1425 ng/L), and feline trypsin-like immunoreactivity (fTLI) (RI: 12 - 82 ug/L) were determined. As per the reference laboratory, a diagnosis of pancreatitis is considered with fPL values > 3.5 ug/L or fTLI values > 100 ug/L. EPI was considered with fTLI values ≤8 µg/L.

Immediately after euthanasia a gross post-mortem examination was conducted. Samples from the body and both limbs of the pancreas were removed with at least 6 sections taken, 2 from each lobe or from the body submitted in separate containers, with a few cases where the pieces of pancreas were submitted in a single container. These samples were labeled, fixed in 10% neutral buffered formalin for at least 48-hours, embedded in paraffin, cut and 5 micron sections were stained with hematoxylin and eosin for routine light microscopy evaluation.

Two board certified pathologists, blinded to all other results, reviewed, described, and graded the histological sections using the classification suggested by De Cock et al. (2007).(16) Excel software was used to calculate the median and interquartile range (IQR) of the continuous data.

**Results**

**Animals**

Twenty young adult cats (17 intact males, 3 intact females) were included in this study. None of the eight cats that were recruited for the praziquantel study were removed due to complications of treatment and good appetite and normal activity were recorded throughout the
duration of this study. All cats tested positive for *Platynosomum* spp. egg on standard parasitological fecal testing, in addition histopathology confirmed biliary fluke infestation.

**Histopathology**

Table 1 depicts the histopathology of the twenty cats that were necropsied. Case 12 had evidence of mild, acute pancreatitis based on the presence of small intralobular lesions containing a few neutrophils within the inflammatory cell infiltrate. Eleven cats, including case 12, had mild chronic pancreatitis characterized by interlobular lymphocytic inflammation and 13 cats had mild periductular fibrosis. Mild peri-pancreatic fat inflammation was noted in case 3, nodular hyperplasia in cases 8 and 13 with minimal amyloid deposition within pancreatic islets also seen in case 13. Chronic mild to moderate interstitial lymphocytic and eosinophilic pancreatitis was detected in the right pancreatic lobe of case 14, which also exhibited a moderate hyperplastic and eosinophilic lymphadenitis within regional pancreaticoduodenal lymph nodes. Pancreatic lesions in this animal were interpreted as significant, even though they involved less than 20% of the affected lobe (Figure 1 and 2), and the result of platynosomosis. Cholangitis, mild to severe, was confirmed in all 20 cats and cholecystitis, mild and moderate severity, in 18 of the cats.

**Ultrasonographic evaluation**

The left limb, right limb, and the body of the pancreas were visualized in 14, 14, and 13 cats respectively of the 20 cats examined. The median thickness and IQR of the left limb, right limb, and body of the pancreas was 8.1 mm (IQR: 6.2 mm; 8.5 mm; n = 14), 4.95 mm (4.1 mm; 6.7 mm; n = 14), and 6.6 mm (4.3 mm; 7.4 mm; n = 13) respectively. One of the twenty cats (case 14) had a thickened (> 1 cm) pancreas, with the left pancreatic limb measuring 10.8 mm. The margination of the right or left limb was described as irregular in two and five cats.
respectively, two of which also had irregular margination of the pancreatic body. An additional four cats had irregular margination of the body without abnormal limb margination.

Echogenicity of the left limb, right limb, and pancreatic body was described as normoechoic in eight, nine, and five cats respectively; hyperechoic in two, three, and two cats respectively; hypoechoic in four, two, and six cats respectively. Peripancreatic fat was considered normoechoic in all cats without evidence of free fluid. The median pancreatic duct was 1.3 mm (0.9 mm; 1.65 mm; n = 11) with a diameter of greater than 10 mm in seven cats.

Based on the ultrasonographic criteria of pancreatitis, 13 cats had a score of 1 and 7 cats had a score of 0; thus none of the cats were considered to have pancreatitis based on ultrasound results (Table 1).

*fPL, cobalamin, folate, and fTLI results*

With few exceptions, the concentrations of fPL, cobalamin, folate, and fTLI for the 20 cats in this study were within the normal reference range (Table 1). One cat (case 20) had increased fPL (22.7 µg/L) and high normal fTLI (79.1 µg/L) concentrations consistent with acute pancreatitis as well as low folate concentrations. Four cats (cases 5, 6, 8 and 17) had cobalamin concentrations below reference range. Five cats (cases 1, 8, 17, 18, and 20) had low folate, and six cats (cases 4, 9, 13, 14, 15, and 16) had increased folate concentration. Case 6 had a fTLI concentration below published reference range (10.2 µg/L) but not within the diagnostic reference range (<8 µg/L) for EPI, in addition to a low cobalamin concentration (163 ng/L).

**Discussion**

In this population of twenty clinically healthy, young adult, feral cats diagnosed with platynosomosis and confirmed cholangitis and cholecystitis, pancreatic histopathology supported the diagnosis of mild acute pancreatitis in one cat and mild chronic pancreatitis in 11 (55%). Five
of these cats also had abnormal clinical chemistry results that may have been related to pancreatic disease yet none of the cats had significant ultrasound changes. While abdominal ultrasonographic parameters of pancreatitis are not well defined, particularly in the case of chronic forms, abdominal ultrasonography remains a practical non-invasive imaging modality used for antemortem diagnosis of pancreatitis. Fortunately age-related pancreatic changes in echogenicity and width are not significant in cats as they are in humans;(30) thus there was no need to account for age in our study. In this study, while pancreatic changes were visible, none of the cats had sufficient criteria (pancreatic limb thickening, loss of margination and peripancreatic fat hyperechogenicity) to fulfill the ultrasonographic diagnosis of pancreatitis. Hyperechoic peripancreatic fat, considered a sensitive index of pancreatitis in cats (68%) (13), was not found in any of the cats in this study. Unfortunately the often multifocal histological distribution of pancreatitis does not aid the ability to diagnose pancreatitis by ultrasonography.(13). Ferreri et al (2003) showed that 54% and 46% of cats with acute and chronic pancreatitis respectively had unremarkable ultrasonographic changes of the pancreas.(20) In addition ultrasonographic changes were unable to distinguish acute from chronic pancreatitis. More recently, Oppliger et al (2014), found similar results in that 39% of cats diagnosed with pancreatitis based on fPL had unremarkable ultrasonographic changes and an agreement between fPL concentration and histopathology was not found.(31) The current test of choice for non-invasive diagnosis of pancreatitis is the fPL concentration (21). In our study, only one cat had an fPL concentration in the range consistent with pancreatitis. In this cat the pancreas was not detectable with ultrasonography and the only noticeable pathology was mild fibrosis. Eleven cats had histopathological changes of chronic pancreatitis. One of these cats, case
6, had abnormal concentrations of both fTLI (10.2 µg/L) and cobalamin (163 ng/L) suggesting a diagnosis of EPI which is assumed to be a consequence of end-stage chronic pancreatitis in the feline species. Mild lymphocytic inflammation and periductular fibrosis was found on pancreatic histopathology. The history of this cat is unknown, but the cat’s body condition score was graded as 2.5/5 (4/9) (32) with a body mass of 2.75 kg, which is considered underweight for an adult male domestic shorthair. However, without the clinical history of chronic enteropathy, the diagnosis of EPI remains equivocal in this individual.

The potential multifocal nature of pancreatitis and small sample sizes are possible reasons that histopathological lesions could have been missed. The lack of a control group is another limitation to the study. Since ultrasonography was not performed by a boarded radiologist, the sensitivity for this procedure could have been influenced by operator experience. Lastly, all cats included in this study were FIV positive which could have potentially affected pancreatic pathology, therefore the results of this study do not necessarily apply to FIV negative cats.

Except for the one case, case 14, with chronic periductal eosinophilic inflammatory cell infiltration and fibrosis, likely the result of platynosomosis, microscopic findings in the pancreas of these Platynosomum spp-infected cats were subtle, non-specific background lesions interpreted as clinically insignificant and not likely related to platynosomosis. Thus, our findings suggest that Platynosomum spp.-induced pancreatic lesions in cats with platynosomosis are rare. While one cat, case 20, had serological indices consistent with pancreatitis based on fPL concentration, and another cat, case 6, had suspected EPI based on a combination of low fTL and cobalamin concentrations, histopathology did not correlate with the diagnoses in either cat. The significance of hypocobalaminemia in four cats with platynosomosis is not yet known but warrants screening of newly diagnosed cases of platynosomosis.
Funding and conflict of interest

Partial funding for this study was provided under a grant from the National Center for Veterinary Parasitology at Oklahoma State University. The authors declare that there is no conflict of interest.

Acknowledgements:

The authors would like to acknowledge Dr Gilda Rawlins who assisted with ultrasonography of several cats, and RUSVM students, Kathleen Neuville and Chele Lathroum who harvested the pancreas at the time of necropsy. In addition, we would like to thank David Hilchie for processing the tissue for histopathology.

References:


Table 1. The severity of cholangitis and cholecystitis determined by histopathology, pancreatic score based on ultrasound, histopathology, and serological testing in 20 cats diagnosed with *Platynosomum* sp. infections. A cumulative score ≥3 was consistent with an ultrasonographic diagnosis of pancreatitis. Feline pancreatic lipase immunoreactivity, cobalamin, and fTLI concentrations were reported as abnormal if they were outside the laboratory reference ranges.
Figures

Fig 1. Chronic interstitial pancreatitis in case number 14. Numerous eosinophils are present within the inflammatory cell infiltrate. Hematoxylin and eosin. Bar: 100 microns.

Fig 2. Chronic pancreatitis in case number 14. Eosinophils are a predominant feature within the periductal and interstitial inflammatory cell infiltrate. Hematoxylin and eosin. Bar E, 40X.
Manufacturer details:

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

b Pfizer Inc. New York, NY

c Buprenex, Reckett Benckiser Healthcare, Hull, England

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

e Euthasol, pentobarbitone sodium, Virbac, Fort Worth, Texas, USA

f Esaote, MyLab™, Genoa, Italy

g Oster® clippers, USA

h GI panel, IDEXX Laboratories, Westbrook, Maine, USA