Candida albicans cholecystitis with associated hepatitis in a cat

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Abstract

Case summary A 3-year-old male neutered domestic shorthair cat was presented for vomiting, inappetence and icterus. Biochemical results and ultrasonographic findings were consistent with cholestasis and possible biliary obstruction. A diagnosis of *Candida albicans* cholecystitis with associated hepatitis was made following cytologic examination and fungal culture. Progressive hyperbilirubinemia and hepatic encephalopathy were ultimately fatal.
Relevance and novel information To our knowledge, this is the first report of biliary candidiasis diagnosed by cytologic examination of a cholecystocentesis sample in a domestic animal with no evidence of immunodeficiency. Additionally, this is the first reported case of fungal cholecystitis with associated white bile syndrome due to obstructive cholestasis, without an overt gall bladder mucocele.

Keywords: Cholecystitis; hyperbilirubinemia; candidiasis; icterus

Introduction

Candida species are normal flora of the alimentary, upper respiratory, and lower urogenital tracts of both humans and animals worldwide. While infection has been reported in humans secondary to disruption of the mucosal barrier, antibiotic-induced overgrowth and immunosuppression, primary infection and secondary complications have rarely been reported in cats and dogs.[1–4]

The mechanisms by which *Candida* species adhere to and colonize mucosal surfaces have been well described;[1] however, in order to cause disease, normal host defense mechanisms must be impaired. Host conditions that increase the risk of systemic candidiasis include previous glucocorticoid therapy, antibiotic therapy, gastrointestinal ulceration associated with previous non-steroidal anti-inflammatory drug (NSAID) therapy, immunosuppressive drugs, indwelling or intravenous catheters, urinary catheters and diabetes mellitus.[2–4] Reported cases of candidiasis in immunocompromised dogs and cats have involved the lower urinary tract and peritoneal cavity,[2,3] and, more recently, the gastrointestinal tract of an immunocompetent cat.[5] This case report details the first known case of *Candida albicans* cholecystitis in a cat without evidence of immunodeficiency.

Case description

A 3-year-old male neutered domestic shorthair cat was referred to our institution for investigation into the cause of an acute onset of icterus. The patient reportedly had no past significant medical history, was previously retrovirus negative, and lived both indoors and outdoors. The cat was up to date on vaccinations but was not receiving any heartworm or flea or tick preventatives. In the 24 h preceding presentation, the patient developed acute vomiting, inappetence, lethargy and icterus. On presentation the patient had a dull mentation, but was responsive to stimuli and had normal vital parameters.

A complete blood count (CELL-DYN 3500; Abbott Diagnostics) was unremarkable. A serum chemistry panel (AU480; Beckman Coulter) and urinalysis (VetStix 11)

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revealed a marked hyperbilirubinemia (12.2 mg/dL; reference interval [RI] 0–0.9 mg/dL) with bilirubinuria (6 mg/dL), moderately increased alkaline phosphatase (ALP) activity (205 U/L [RI 0–60 U/L]), and moderate hypercholesterolemia (337 mg/dL [RI 80-250 mg/dL]) indicative of cholestasis. There was evidence of hepatocellular injury given the markedly increased alanine aminotransferase (ALT) activity (1863 U/L [RI 0–100U/L]), and moderately increased aspartate aminotransferase (AST) activity (586 U/L [RI 0–60 U/L]). There was a mild hyponatremia (144 mEq/L [RI 148–160 mEq/L]) with a disproportionate moderate hypochloremia (104 mmol/L [RI 115–126 mmol/L]) indicative of fluid losses and a hypochloremic metabolic alkalosis, which was attributed to the patient's vomiting. A coagulation panel (Amelung KC4A; Sigma Diagnostics) revealed a mildly prolonged partial thromboplastin time (23.3 s [RI 10–17 s]) and normal prothrombin time. Serologic assessment of feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) were negative (SNAP FIV/FeLV/HW Triple Test; IDEXX).

Initial abdominal ultrasonography was performed within 24 h of admission and revealed variably organized anechoic-to-hyperechoic material within the gall bladder lumen (Figure 1). The path of the common bile duct could not be followed to the duodenal papilla owing to interference caused by gastrointestinal gas shadowing, as well as inflammation of the surrounding mesentery resulting in attenuation of the ultrasound beam. The left limb of the pancreas was hypoechoic, with adjacent hyperechoic fat. No effusion or free gas was noted, and the remainder of the abdomen was unremarkable. Thoracic radiographs were within normal limits.

The patient was started on antimicrobial therapy consisting of ampicillin sulbactam (22 mg/kg IV q8h [Unasyn; Pfizer]) and enrofloxacin (5 mg/kg IV q24h [Baytril; Bayer DVM]), as well as ondansetron (0.5 mg/kg IV q12h [Ondansetron Accord; Accord Healthcare]) for antiemetic support. Buprenorphine (0.01 mg/kg IV q8–12h [Buprenex (buprenorphine hydrochloride) Injectable; Fresenius Kabi USA]) was also added for analgesia. Forty-eight hours after initial presentation, the patient's hyperbilirubinemia worsened (23 mg/dL); however, clinical improvement was noted as the patient appeared brighter, comfortable on abdominal palpation and eating well. Continued hospitalization was recommended; however, the owners elected to take the patient home for continued monitoring.

Two days later, the patient was re-presented for repeat blood work and evaluation for anorexia. A serum chemistry panel revealed progressive hyperbilirubinemia (26.4 mg/dL) and a worsening hypochloremic metabolic alkalosis (chloride 99 mmol/l, sodium 143 mEq/l), and hypokalemia (3.2 mEq/L [RI 3.5–5.5 mEq/L]), likely due to anorexia. Repeat abdominal ultrasound revealed mostly hyperechoic, organized material against an interface of anechoic content within the gall bladder lumen (Figure 1). The cystic duct was abnormally dilated at 2.9 mm, and the common bile duct was tortuous and difficult to follow. The distal portion of the common bile duct measured 5.0 mm at its termination, where normal feline common bile duct diameter is <4.0 mm.[6] The previously noted pancreatic changes had resolved.

The following day the patient was sedated with butorphanol (0.2 mg/kg IV [Dolorex; Merck Animal Health]) and propofol (5 ml/kg IV [PropoThesia; Henry Schein Animal Health]), and general anesthesia was maintained with isoflurane and oxygen. Ultrasound-guided liver aspirates and cholecystocentesis were performed using a 22 G 1 ½ inch needle, followed by esophagostomy tube (e-tube) placement (14 Fr Mila). The fluid obtained from the cholecystocentesis was grossly white and opaque. Esophagostomy tube placement occurred without incident, and the patient was started on one-third of its resting energy requirement via the e-tube with Clinicare Nutritional Support (Abbott). The following morning, a nematode of unknown type (presumed *Ollanulus tricuspis* or *Physaloptera* species) was discovered in the lumen of the e-tube. A fecal sample was unable to be obtained for parasitic evaluation, and the patient was discharged with a 3 day course of fenbendazole (Panacur; Merck).

The Wright's stained smear obtained from the cholecystocentesis lacked the typical cytologic appearance of bile, which is normally acellular to poorly cellular with a granular-to-amorphous purple/gray background.[7,8] Instead, the background had a hazy proteinaceous-to-mucoid quality with a moderate amount of blood. Leukocytes consisting mostly of mildly degenerate neutrophils appeared increased over the expected contribution from blood. Low numbers of round-to-oval,
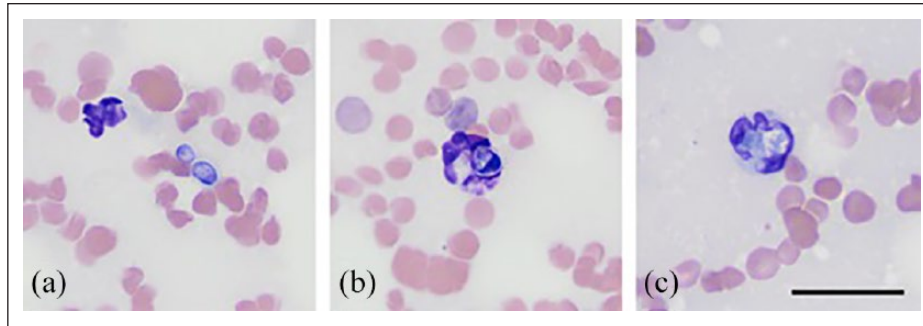

Figure 1 Representative ultrasound image of variably organized hyperechoic and anechoic debris within the gall bladder (GB) lumen

Figure 2 Representative image of a direct smear of fluid obtained from a cholecystocentesis. (a) Mildly degenerate neutrophil with two extracellular round-to-oval, approximately 2 x 5–7 µm, deep-blue yeast organisms with a thin clear halo. (b) Mildly degenerate neutrophil containing a phagocytized yeast organism. The morphologic appearance of the yeast organisms was suggestive of *Candida* species, which was later confirmed on fungal culture. (c) Non-degenerate neutrophil containing phagocytized amorphous blue material, suggestive of mucin. Wright's stain, X 100 objective, bar = 20 µm

approximately 2 x 5–7 µm, deep-blue yeast organisms with a thin clear halo were present extracellularly and rarely phagocytized within neutrophils (Figure 2a,b). No pseudohyphae or hyphal forms were observed. No biliary epithelial cells were present. Rare neutrophils appeared to contain phagocytized blue material, suggestive of mucin (Figure 2c). The cytologic interpretation was mild suppurative inflammation with intralesional yeast organisms. The morphologic appearance of the yeast organisms was suggestive of *Candida* species and fungal culture was recommended for confirmation.

The Wright's stained smears of the liver aspirate were moderately cellular consisting of predominantly hepatocyte clusters, moderate numbers of small to moderately sized clusters of cuboidal-to-low columnar epithelial cells, interpreted as biliary in origin (Figure 3), and moderate numbers of inflammatory cells. The hepatocytes ranged from well-differentiated cells to those that displayed higher nuclear-to-cytoplasmic ratios, increased cytoplasmic basophilia, binucleation and mild-to-moderate anisokaryosis. The atypical hepatocytes were often present in disorganized clusters with streaming noted, suggestive of fibrosis. The hepatocytes contained low numbers of clear, discrete vacuoles consistent with lipid, and small amounts of fine, granular, blue–green lipofuscin pigment. The biliary epithelial cells had a round nucleus with coarsely stippled chromatin and 1–2 indistinct nucleoli. They displayed minimal anisocytosis and anisokaryosis, and rare binucleation. The biliary epithelial cells frequently contained small-to-moderate amounts of pale blue cytoplasmic material, suggestive of mucin (Figure 3). Elsewhere on the smear, there were loose streams and tight spirals of pale-to-dark-blue mucinous material, which occasionally contained entrapped hepatocytes and non-degenerate neutrophils (Figure 4). In addition to neutrophils, few plasma cells and macrophages containing non-specific phagocytic debris were

noted, supportive of tissue inflammation (not shown). No fungal organisms or other infectious agents were observed in the smears from the liver aspirates.

The cytologic interpretation was mild-to-moderate mixed (neutrophils, lymphocytes, plasma cells and macrophages) inflammation, mild lipid accumulation and probable biliary hyperplasia. While a biliary adenoma, cystadenoma or well-differentiated carcinoma could not be ruled out, this was considered unlikely given the lack of reported nodular and/or mass lesions in the liver. Biliary hyperplasia was favored given their well-differentiated appearance and the concurrent presence
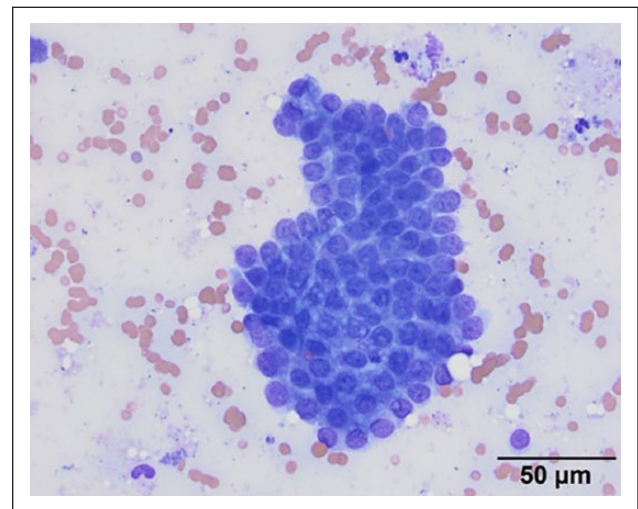

Figure 3 Representative image from a fine-needle aspirate of the liver. A single uniform cluster of cuboidal-to-low columnar epithelial cells, interpreted as biliary in origin. Note the amorphous, pale-blue material, interpreted as mucin, within the cytoplasm of the cells. This material is not a normal finding in biliary epithelium seen as an incidental finding in liver aspirates, and is suggestive of excessive mucus production and/or inspissation. Wright's stain, X 50 objective, bar = 50 µm
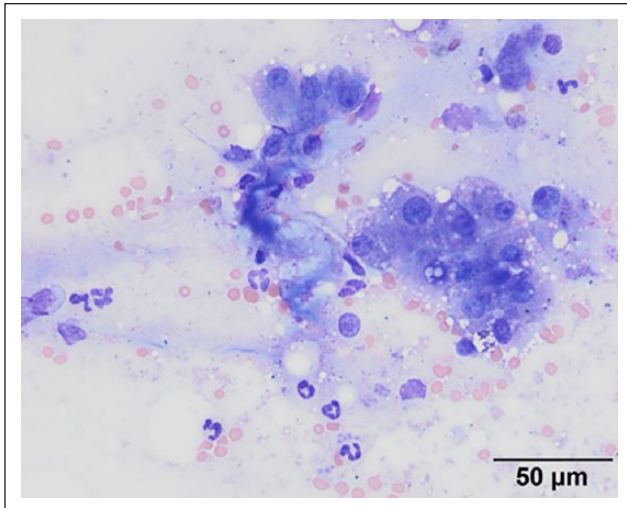
Figure 4 Representative image from a fine-needle aspirate of the liver. Hepatocytes and increased numbers of non-degenerate neutrophils are caught up within loose streams and tight spirals of pale-to-dark-blue mucinous material. This mucin material is an abnormal finding in liver aspirates and was suggestive of 'white bile', as can be seen with gall bladder mucoceles. The hepatocytes are noted to display moderate anisocytosis and anisokaryosis and increased cytoplasmic basophilia, interpreted as a regenerative response to the inflammation. Low numbers of clear discrete vacuoles, consistent with lipid, are also visible within hepatocytes. Wright's stain, X 50 objective, bar = 50 μm

of inflammation.[9] The cytologic atypia noted in the hepatocytes was interpreted as reactive, likely representative of a regenerative response in the liver secondary to the inflammation present. The abundant mucoid material in the sample is an abnormal finding for liver aspirates, and the possibility of 'white bile', as has been reported with gall bladder mucoceles and extrahepatic biliary obstruction, was raised.[10,11] Histopathologic examination was recommended to confirm these findings.

The patient continued to tolerate e-tube feedings well, but started to decline systemically. Ten days after initial presentation the cat was found to be obtunded and hypotensive (systolic blood pressure 40–96 mmHg). Recheck packed cell volume revealed an anemia (20% [RI 24–45%]), and recheck chemistry revealed a progressive hyperbilirubinemia (33.8 mg/dL) and persistent liver enzyme increases (ALT 1486 U/L, AST 663 U/L, gamma-glutamyl transferase 17 U/L, ALP 241 U/L). There was also a newly documented mild fasted hyperammonemia (128 μmol/l [RI 0–100 μmol/l]), that in conjunction with the clinical presentation and chemistry results, was supportive of hepatic encephalopathy. Repeat ultrasound-guided cholecystocentesis was performed without sedation using a 22 G, 1 ½ inch needle in order to obtain a sample for fungal culture. Later that evening the patient experienced cardiorespiratory arrest. The fungal culture grew an isolate of *C albicans* (Cornell University Animal Health Diagnostic Center).

Discussion

To our knowledge, this represents the first report of biliary candidiasis in a companion animal. There was no evidence of immunodeficiency, the patient was retrovirus negative and no underlying cause was identified leading to the candidiasis infection. Historically, the patient was systemically healthy until 24 h prior to presentation, making an undetected predisposing condition unlikely. The cat also lacked a history of antibiotic, NSAID or glucocorticoid administration. While a nematode was discovered in the lumen of the esophagostomy tube, a fecal evaluation was unable to be performed to identify a parasitic infection. It is unclear whether or not this could have been an incidental finding for the patient, or represented underlying gastrointestinal disease.

Possible causes for the patient's candidiasis include intestinal dysbiosis and biliary reflux of intestinal contents. Similar to the pathogenesis of bacterial cholangitis, it is possible that commensal intestinal candida was permitted to reflux through the common bile duct and colonize the patient's gall bladder. Unique to feline anatomy, the common bile duct and pancreatic duct join together prior to emptying into the duodenum at the major duodenal papilla.[12] Additionally, impaired intestinal barrier function may also result in translocation of enteric bacteria to the pancreas and the liver.[13] This has been supported in several studies utilizing both bacterial cultures and fluorescence in situ hybridization to detect enteric bacteria in the pancreas, liver and bile of cats with cholangitis or cholangiohepatitis.[14–16] Compared with dogs, cats also have higher duodenal bacterial counts, which could act to further predispose feline patients to reflux and cholangitis.[17] The presumed nematode discovered in the lumen of the patient's e-tube may have also acted as a risk factor for biliary reflux and subsequent biliary candidiasis.

Candida species can form hyphae, which act as a virulence factor associated with infection, allowing invasion of epithelial cells, which leads to tissue damage.[18] Additionally, the presence of bile acids has been shown to promote *Candida* virulence.[19] *Candida* species virulence may also be increased in cases of altered biliary flow, and, in particular, with obstructive disease processes. It is also possible that the organizing intraluminal gall bladder debris could have represented a developing mucocele, as supported by the abnormal gross appearance of the biliary fluid upon aspiration. White bile syndrome has been reported in patients with extrahepatic bile duct obstruction, in which the biliary epithelium continues to secrete mucus despite stagnant bile flow, due to an obstruction at the level of the cystic duct.[20,21] In humans, the presence of white bile has been associated

with a shorter survival time for patients with malignant jaundice.[22] While mucoceles are rarely reported in cats,[23–25] early mucocele formation causing a biliary obstruction may have led to cholestasis and opportunistic invasion of *Candida* species from the intestinal tract in the patient reported here.

Given the evidence of pancreatitis on initial ultrasound, extrahepatic biliary obstruction could not be ruled out as a cause of the patient's hyperbilirubinemia. While abdominal ultrasound has a low sensitivity for the diagnosis of pancreatitis, ranging from 11–67%, the specificity is consistently higher.[26–28] A recent study revealed that hyperechoic peripancreatic fat was the single ultrasound characteristic with the highest sensitivity for pancreatitis,[29] which was documented in the current patient. Surgical intervention for a cholecystectomy and possible biliary stenting was discussed; however, the owners did not elect to pursue surgical intervention. Ideally, medical management would have been instituted, with antifungal therapy based on susceptibility results. Treatment of candidiasis should involve both specific antifungal drug treatment, as well as management of any underlying immunosuppressive disorders and discontinuation of systemic antimicrobials if indicated.[3,4] Intravesicular treatment with 1% clotrimazole has also been reported in both a cat and a dog with urinary candidiasis;[30,31] however, localized gall bladder therapy has not been described. Susceptibility testing was performed postmortem for the current patient, however no interpretations of antifungal susceptibility were made, as minimum inhibitory concentration values have not been established for C. albicans.

Conclusions

We describe the first report of biliary candidiasis in a domestic animal, as well as the first reported case of fungal cholecystitis with associated white bile syndrome due to obstructive cholestasis. This case highlights the importance of performing cholecystocentesis for cytologic examination in patients with hyperbilirubinemia and cholestasis, especially if treatment with empiric antibiotic therapy has failed. Additionally, while candidiasis has been well documented in immunocompromised patients, this case raises suspicion for the role of the pathogen in primary disease processes.

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