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Case Report

Spontaneous Cholelithiasis in a Squirrel Monkey (*Saimiri sciureus*)

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A mature female squirrel monkey was noted during routine semiannual examinations to have moderate progressive weight loss. Serum chemistry panels revealed marked increases in hepatic enzyme, bilirubin, and bile salt concentrations and hypoalbuminemia. Abdominal ultrasonography revealed echogenic, shadowing debris in the gallbladder, consistent with cholelithiasis. At necropsy, marked thickening and distension of the gallbladder, cystic duct, and common bile duct was noted, and more than 50 irregularly shaped, black gallstones were removed from the biliary tract. Gallbladder tissue, bile, and gallstones cultured positive for *Escherichia coli* and *Proteus* spp., suggesting a brown-pigment gallstone type secondary to a bacterial nidus. Histopathology revealed severe chronic–active diffuse cholecystitis and severe chronic-active hepatic degeneration and necrosis with severe cholestasis. To our knowledge, this report is the first description of spontaneous choleilthiasis in a squirrel monkey.

Cholelithiasis is a common disease, affecting approximately 10% to 15% of adults in the United States.[9,15] Gallstones typically are classified as either cholesterol gallstones or pigment gallstones, depending on their cholesterol content. Spontaneous cases of cholelithiasis have previously been reported in several species of New World primates, including cholesterol gallstones in owl monkeys (*Aotus* spp.) and pigment gallstones in lion tamarins (*Leontopithecus* spp.) and marmosets (*Callithrix* spp.).[2,11] Squirrel monkeys on high-cholesterol diets have historically been used as an experimental model for cholelithiasis.[8,13] We report a naturally occurring case of cholelithiasis in a squirrel monkey that was maintained for 11 y on a standard commercial diet.

Case Report

During routine semiannual physical examination performed under ketamine sedation (10 mg/kg IM), a mature adult female squirrel monkey (*Saimiri sciureus*) was found to be thin, with a poor body condition score and thinning haircoat. The squirrel monkey was acquired as a young adult and pair-housed indoors for 11 y in an AAALAC-accredited facility and maintained in accordance with the *Guide for the Care and Use of Laboratory Animals*.[5] Routine husbandry parameters include a 12:12-h light:dark cycle; controlled humidity, temperature, and ventilation; unrestricted water, and commercial primate chow (Purina 5037, Purina Mills, St Louis, MO) supplemented daily with fresh fruit, vegetables, and a foraging mixture of dried fruit, nuts and cereal. At the time of arrival into the colony, the squirrel monkey was negative for tuberculosis by intradermal tuberculin test and IFNγ serology, seronegative for measles virus, and seropositive for *Herpesvirus saimiri*, *Herpesvirus tamarinus*, and squirrel monkey cytomegalovirus. The squirrel monkey was surgically naïve, with an experimental history of anesthesia for noninvasive imaging studies. All research protocols were approved by the Massachusetts Institute of Technology Committee on Animal Care. At the time of scheduled examination, blood was collected for a CBC and serum chemistry panel, and selected values are shown (Table 1) in comparison with the last known normal values obtained 2 y prior to presentation. Although the CBC results were within normal limits, the serum chemistry panel revealed elevations in hepatic enzymes (ALT, AST, GGT) and mild hypoalbuminemia. Because the animal's appetite and activity levels were observed to be clinically normal, monitoring was elected until the next regularly scheduled examination.

Follow-up examination performed 6 mo later revealed progressive weight loss but no other physical abnormalities (Figure 1). The repeat CBC was within normal limits, and serum chemistry results revealed further increases in cholestatic enzymes (ALP, GGT) and progressive hypoalbuminemia. However, ALT and AST were decreased compared with the initial values, and the animal's serum cholesterol level remained within normal limits. At this time, the problem list included elevated cholestatic enzymes (GGT), elevated hepatocellular leakage enzymes, and weight loss. Differentials for cholestasis included intrahepatic causes, including infection, neoplasia, and cholangiohepatitis, and extrahepatic causes resulting from diseases of the gallbladder, cystic duct, or common bile duct.

Abdominal ultrasonography revealed focal echogenic shadowing debris (approximately 2×3 mm) in the gallbladder, consistent with cholelithiasis. Abdominal radiographs revealed poor serosal detail due to decreased body mass, with no evidence of radiopacity in the expected anatomic location of the gallbladder. Due to the surgical complexity of cholecystectomy or cholecystotomy in such a small species, advanced imaging was requested prior to surgical intervention. Although planned, CT at a neighboring facility was delayed until construction of an appropriately sized

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Table 1. Selected clinical chemistry values in a squirrel monkey with cholelithiasis

	October 2011	October 2013	April 2014	August 2014	Reference range[a]
ALP, IU/L	301	314	602	3415	33–859
ALT, IU/L	121	760	301	374	40–182
AST, IU/L	112	476	223	373	71–201
Creatine kinase, IU/L	432	355	617	ND	0–1995
GGT, IU/L	31	143	830	4043	0–53
Total bilirubin, mg/dL	0.1	0.6	0.3	8.4	0–0.8
Albumin, g/dL	3.1	3.0	2.6	1.9	3.0–4.4
Globulin, g/dL	2.5	3.1	3.4	3.4	2.1–3.7
Total protein, g/dL	5.6	6.1	6.0	5.3	6.0–7.2
Cholesterol, mg/dL	184	232	216	207	142–240
BUN, mg/dL	19	17	17	17	19–39
Creatinine, mg/dL	0.5	0.7	0.6	ND	0.5–1.1
Glucose, mg/dL	85	82	106	62	46–126
Ca^{2+}, mg/dL	9.1	8.5	8.3	ND	8.2–9.8

ND, not done.
Chemistry values reported from a commercial veterinary laboratory; the last known normal values (October 2011) are included for comparison.
[a]Reference ranges obtained from the International Species Information System Reference Ranges for *S. sciureus*, created March 2002. http://www2.isis.org/support/MEDARKS/Pages/Reference%20Ranges.aspx (website accessed 29 June 15)

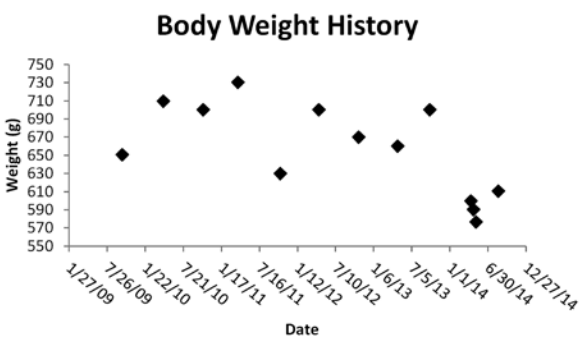

Figure 1. Abbreviated history of body weight over time, showing marked decline.

cradle. In the interim period, the squirrel monkey was started on a daily, oral hepatic antioxidant supplement containing S-adenosylmethionine and silybin (22.5 mg and 2.25 mg, Nutramax Labs, Lancaster, SC). Repeat liver chemistries revealed marked increases in ALP, GGT, and total bilirubin; persistent elevation of ALT and AST; and progressive hypoalbuminemia (Table 1). Repeat abdominal ultrasonography revealed an enlarged gallbladder containing echogenic debris and a dilated cystic duct containing multiple discrete hyperechoic shadowing objects (Figure 2).

At 12 h prior to the CT scan, 250 mg of compounded iopanoic acid was administered orally as a hepatobiliary contrast agent. The squirrel monkey was sedated with ketamine (5 mg/kg IM) for intravenous catheter placement and maintained on inhalant isoflurane and oxygen during imaging. However, respiratory arrest occurred prior to completion of the scan, and cardiopulmonary resuscitation attempts were unsuccessful.

Pathology. At necropsy, inspissated, purulent-appearing bile was aspirated from the gallbladder, which was markedly dilated. Incision into the biliary tract revealed numerous (more than 50), irregularly shaped, hard, black stones in the gallbladder, cystic duct, and common bile duct (Figures 3 and 4). Grossly, the liver was shrunken and firm, with a diffusely irregular capsular surface. Tissue samples were collected and fixed in 10% neutral buffered formalin prior to processing, paraffin-embedding, and sectioning. Tissue sections (thickness, 5 µM) were stained with hematoxylin and eosin and Masson trichrome. Histopathology revealed severe chronic–active diffuse cholecystitis as well as severe diffuse hepatic degeneration and necrosis with chronic–active cholangiohepatitis, bile duct hyperplasia, and cholestasis (Figures 5 and 6).

Gallbladder tissue, bile, and gallstones were submitted for aerobic and anaerobic cultures. Bile was aspirated by using a sterile needle and syringe and plated by using sterile swabs onto chocolate agar, trypticase soy agar with 5% sheep blood, MacConkey agar, and CDC anaerobic agar. Aerobic plates were incubated at 37 °C, 5% CO_2 for 24 h, whereas CDC plates were incubated in an anaerobic chamber for 48 h. The swabs and remainder of the bile sample were incubated in thioglycollate broth at 37 °C for 24 h and replated onto the media listed. Individual colonies were isolated on blood agar and identified by using the Analytical Profile Index identification system (API 20 E and API 20 Strep, bioMérieux, Durham, NC). Similar culture techniques were used on gallbladder tissue and gallstones after manual disruption with a sterile mortar and pestle. The anaerobic culture was negative; aerobic cultures isolated *Enterococcus faecalis*, *Escherichia coli*, and *Proteus* spp. from bile and gallstones and *E. coli* and *Proteus* spp. from gallbladder tissue.

DNA was extracted from homogenized gallbladder tissue (High Pure PCR Template Preparation Kit, Roche Life Science, Indianapolis, IN) according to manufacturer instructions. *Helicobacter*-genus level primers (forward, C97; reverse, C05) were used for amplification of a 1.2-kb PCR product before detection by electrophoresis (on a 1% agarose gel), ethidium bromide staining, and visualization with UV light as previously described.[4,7] By using a glass tissue grinder, small section of gallbladder tissue was homogenized in *Brucella* broth containing 20% glycerol. A 1-mL portion of the tissue suspension was gently pushed through a 0.65-µm syringe filter, and the homogenate was streaked onto

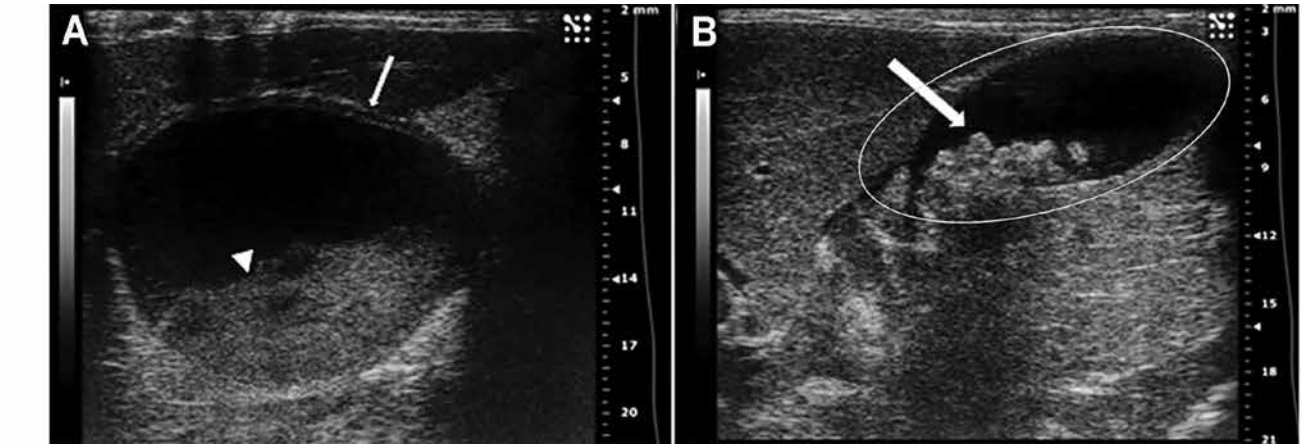

Figure 2. Abdominal ultrasonography. (A) Gallbladder contains echogenic material (arrowhead). A hypoechoic region within the gallbladder wall is suggestive of intramural edema (arrow). (B) The cystic duct (circle) is dilated with multiple echogenic, shadowing structures consistent with choleliths (arrow).

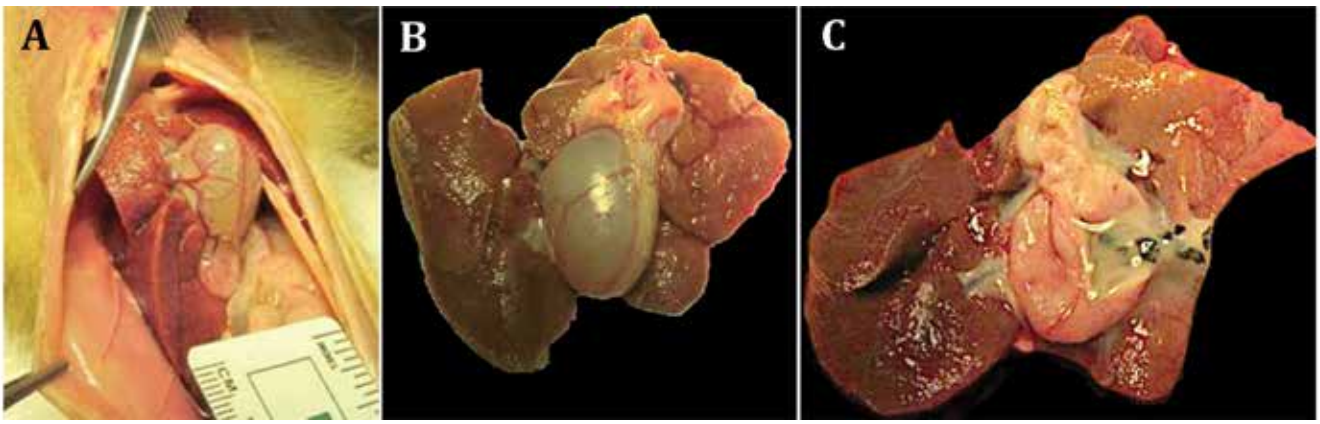

Figure 3. Gross necropsy images. (A) Gallbladder and liver in situ. Note the cobblestone texture of the liver and the marked distension of the gallbladder. (B) Excised hepatobiliary tree, showing the dilated cystic duct and common bile duct. (C) Incision into the gallbladder revealed purulent bile and many small, irregularly shaped, black choleliths.

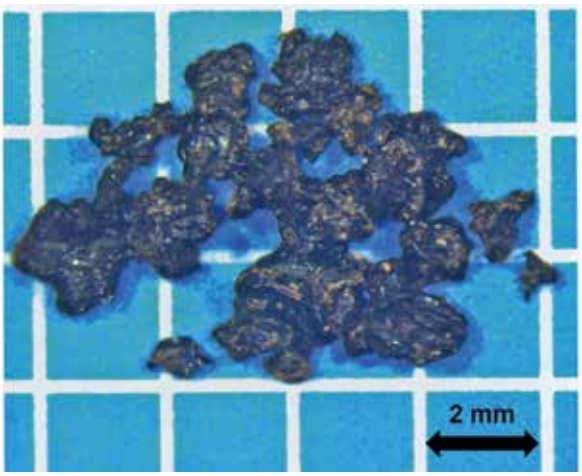

Figure 4. Photomicrograph of gallstones. Each grid division represents 2 mm. Photo provided by the Louis C Herring Laboratory.

a trypticase soy agar (with 5% sheep blood) plate (Remel Laboratories, Lenexa, KS); in addition, 0.1-mL samples of the tissue suspension were directly added onto CVA plates, which contain cefoperzone, vancomycin, and amphotericin B (Remel). The cultures were incubated at 37 °C under microaerobic conditions in vented jars containing N_2, H_2, and CO_2 (80:10:10). Plates were examined every 3 to 4 d for a maximum of 3 wk to isolate *Helicobacter*-like colonies. Gallbladder PCR analysis and cultures performed for *Helicobacter* spp. were negative.

Calculi were submitted to a commercial reference laboratory (Louis C Herring Laboratory, Orlando, FL)for microchemical testing, polarized light analysis, and infrared spectrum analysis. The gallstone composition could not be definitively determined, because testing results were not consistent with calcium bilirubinate, calcium carbonate, or cholesterol composition, and the infrared spectrum did not match known reference spectra. We contacted 2 alternative commercial stone-analysis laboratories regarding additional analysis, but neither accepted samples from NHP. Ex vivo microCT scans of the gallstones found a radiographic density similar to water, confirming that in vivo radiography was unlikely to image the gallstones.

Discussion

The pathogenesis of gallstone formation is still not fully understood, but gallstones arise after supersaturation and precipitation

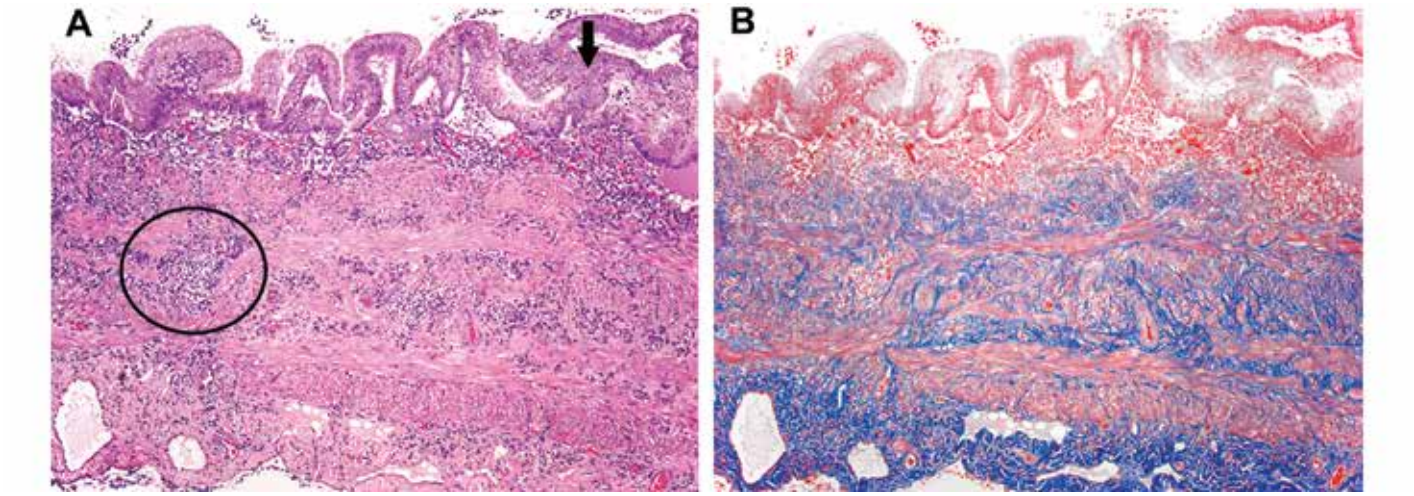

Figure 5. Gallbladder histopathology. A. Section showing chronic–active cholecystitis as characterized by hyperplastic mucosa (arrow), transmural mixed inflammation (circled), and ectatic lymphatics (arrowhead). Hematoxylin and eosin stain. (B) Marked fibrous connective tissue is present. Trichrome stain; magnification, 10×.

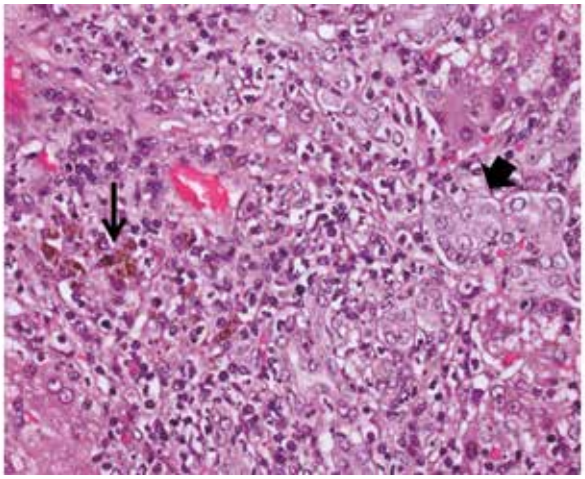

Figure 6. Histopathologic image of the liver, demonstrating bile duct hyperplasia (thick arrow) and inflammatory cell infiltration. Note the brown granular material within macrophages, representing intracytoplasmic bile (thin arrow). Hematoxylin and eosin stain; magnification, 40×.

of biliary solutes.[15] In humans, cholesterol gallstones are the most common type, representing 70% of cases in the Western world.[15] Risk factors for cholesterol gallstones include age, obesity, dietary cholesterol levels, and sex, with women more commonly affected than men.[9] Diets high in cholesterol can predispose to cholesterol gallstone formation when the normal emulsification role of bile is overwhelmed by excess excretion of hepatic cholesterol.[15] In addition, causes of biliary stasis, including infection, hypomotility, and biliary obstruction can lead to pigment stone formation. Pigment stones are composed primarily of calcium bilirubinate and are classified as black or brown pigment stones.[14] Black pigment stones are formed under sterile conditions and arise from the precipitation of bilirubinate secondary to hemolysis or hepatic cirrhosis[1,6,14]. In the absence of hyperbilirubinemia, black pigment stones can arise spontaneously in germ-free Swiss Webster mice with impaired gallbladder motility and response to intestinal cholecystokinin.[16] Brown pigment stones typically are associated with infection, because hydrolytic enzymes from enteric bacteria liberate fatty acids to precipitate with calcium bilirubinate.[15] Bile cultures from patients with biliary disease have revealed *E. coli*, streptococci, enterococci, *Klebsiella* spp., *Pseudomonas* spp., and *Proteus* spp. as the most commonly isolated aerobic species and *Clostridium* and *Bacteroides* spp. as the most commonly isolated anaerobic species.[12] Polymicrobial infections are reported frequently, with *E. coli* as the most commonly isolated bacterial species.[14] Brown pigment stones typically are distinguished from black pigment stones morphologically by their softer nature and alternating concentric layers on cross section.[6,14]

Squirrel monkeys have been historically used as models of cholesterol cholelithiasis when fed lithogenic diets. A variety of diet-supplementation protocols have been evaluated, but dietary cholesterol supplementation (0.9 mg/kcal) reliably induces cholesterol gallstone formation.[13] Unlike the situation in humans, there is no sex-associated predilection to cholesterol cholelithiasis in squirrel monkeys, with males and females affected equally. In addition, although the administration of oral chenodeoxycholic acid is used to dissolve cholesterol gallstones in human patients, this practice does not protect against cholesterol gallstone formation in squirrel monkeys.[13]

In a report of 6 cases among 4 callitrichid species, the most common clinical findings associated with cholesterol gallstone formation were thin body condition, chronic weight loss, lethargy and weakness.[11] The most common hematologic abnormality was hyperbilirubinemia, which was reported in 4 cases. Of these 6 cases, 3 were treated surgically with poor outcome due to postsurgical complications and weight loss. Of the 3 nonsurgically treated animals, one died acutely 1 mo after diagnosis, and the remaining 2 were euthanized for weight loss at 1 y and 3 y after diagnosis. Choleliths isolated from these callitrichids were diverse in composition and radiopacity; 2 of the 5 stones characterized were radiolucent in appearance and were composed of at least 60% miscellaneous bile pigments. Furthermore, 3 gallstones were cultured (2 at the time of surgery and one nonsurgical case at necropsy), and all 5 gallstones analyzed were characterized as pigment stones.

Similar to the callitrichid cases, the main clinical presentation in our case was chronic weight loss. Although cirrhosis in humans is

a well-known risk factor for pigment stones, whether the chronic–active hepatitis in the current case was a predisposing factor or sequelae to gallstone formation is difficult to ascertain.[1,3] Although our animal was seropositive for 3 squirrel monkey viruses (*Herpesvirus saimiri*, *Herpesvirus tamarinus*, and squirrel monkey cytomegalovirus), none of these has been associated with persistent viral hepatitis. Experimental infection with hepatitis A has been reported, but natural causes of hepatitis in squirrel monkeys are not identified in the literature.[10] The relatively low magnitude of hepatic transaminase elevation and lack of viral inclusion bodies on liver histopathology make a viral etiology unlikely in this case. Translocation of intestinal microflora as a bacterial cause of hepatitis cannot be ruled out, but the etiology of initial gallstone formation is unknown.

Although the composition of the gallstones in the squirrel monkey we report could not be definitively identified, the lack of a high-cholesterol diet and an infrared spectrum inconsistent with cholesterol stones make it unlikely that these gallstones were predominantly cholesterol in composition. The presence of enteric bacteria in the stones, bile, and gallbladder tissue suggest that these stones, despite their atypical morphologic appearance, are most consistent with brown pigment stones. Although spontaneous cholelithiasis is uncommon in NHP, chronic weight loss and elevated cholestatic enzymes are key clinical findings. Elevations in hepatic transaminases should be monitored regularly, especially when unexpected in conjunction with experimental manipulation. To our knowledge, this case represents the first report of spontaneous cholelithiasis in a squirrel monkey.

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