2,8-dihydroxyadenine uroliths in a dog

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Case Description—A 43-kg (95-lb) 4-year-old neutered male mixed-breed dog was evaluated because of a 2-day history of dysuria.

Clinical Findings—Radiography and ultrasonography revealed hydronephrosis, hydroureter, and radiolucent, hyperechoic uroliths in the right kidney and ureter and the urinary bladder. Serum bile acids concentration was within the reference interval.

Treatment and Outcome—The uroliths in the bladder and right ureter were surgically removed and submitted for analysis. They were initially identified as urate uroliths; however, results of further analysis indicated uroliths were composed of 2,8-dihydroxyadenine (2,8-DHA), and 2,8-DHA was identified in a urine sample of the dog. Allopurinol was prescribed for the dog, and a purine-restricted diet was recommended.

Clinical Relevance—2,8-DHA uroliths are extremely rare in humans and dogs. Such uroliths may be underdiagnosed in humans because of variability of clinical signs and difficulty in differentiating 2,8-DHA and urate uroliths and crystalluria. Uroliths composed of 2,8-DHA may be misdiagnosed as urate uroliths in dogs. (*J Am Vet Med Assoc* 2012;241:1348–1352)

A 43-kg (95-lb) 4-year-old neutered male mixed-breed dog (identified by the owner as an American Indian dog [related to Siberian Huskies, Alaskan Malamutes, Chinooks, and other large breeds of dogs]) was evaluated at the Peel Veterinary Clinic with a 2-day history of dysuria. Severe bilateral hip dysplasia had been diagnosed in the dog 32 months prior. The dog had been fed a commercially available dry food formulated for dogs with osteoarthritis[a] and table scraps. The dog was not receiving any medications. Abdominal radiography revealed a fully distended urinary bladder but no uroliths. A urinary catheter was passed into the bladder via the urethra without resistance. A urine sample was collected; the sample was brown and cloudy, had a pH of 5 and a urine specific gravity of 1.028, and no crystals were observed during microscopic examination of urine sediment. Culture and susceptibility testing were not performed. Treatment with amoxicillin-clavulanic acid[b] (12.8 mg/kg [5.8 mg/lb], PO, q 12 h for 15 days) and tramadol hydrochloride (2.3 mg/kg [1.0 mg/lb], PO, q 12 h for 10 days) was started. Results of serum biochemical analyses indicated an elevated creatinine concentration (132 µmol/L; reference interval, 27 to 124 µmol/L). Values of all other serum biochemical analysis variables were within the reference intervals, including urea (9 mmol/L; reference interval, 2 to 9 mmol/L) and albumin (38 g/L; reference interval, 25 to 44 g/L) concentrations. The dog was discharged from the hospital that same day.

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Abbreviations

2,8-DHA	2,8-dihydroxyadenine
APRT	Adenine phosphoribosyltransferase
HPRT	Hypoxanthine phosphoribosyltransferase

Four days later, the dog was readmitted to the veterinary clinic for evaluation of an acute inability to urinate. Resistance was felt during an attempt to pass a urinary catheter into the bladder via the urethra. The urinary catheter was removed, and a small urolith was found lodged in the tip. This urolith was submitted to a laboratory[c] for analysis. Urethral catheterization was repeated, and 2 more uroliths were removed. The uroliths were green, irregular in shape, and friable. Results of urinalysis indicated a urine specific gravity of 1.033, a urine pH of 6.5, and numerous clusters of round, gold-colored crystals with radiating striations, which were suspected to be urate crystals. Results of laboratory analysis indicated the urolith was 100% ammonium urate. Because no uroliths had been detected via radiography, the dog was admitted to a specialty veterinary hospital 4 days later for further evaluation, including ultrasonography of the urinary system.

Signs of pain were detected in the caudal aspect of the abdomen of the dog during physical examination at the referral hospital; no other abnormalities were detected. Abdominal ultrasonography revealed the urinary bladder was moderately distended and contained several small uroliths (diameters, ≤ 0.49 cm; **Figure 1**). A small urolith (diameter, 0.8 cm) and mild to moderate hydronephrosis were detected in the right kidney (**Figure 2**). A focus of 1 or more uroliths (1.6 × 1.06 × 2.17 cm) was detected in the proximal aspect of the right ureter 1.20 cm distal to the kidney (**Figure 3**). The right ureter was dilated (diameter, 0.56 cm) along the entire length (from the right kidney to the urinary bladder). The right renal pelvis was dilated (1.58 × 0.78 cm). The combina-

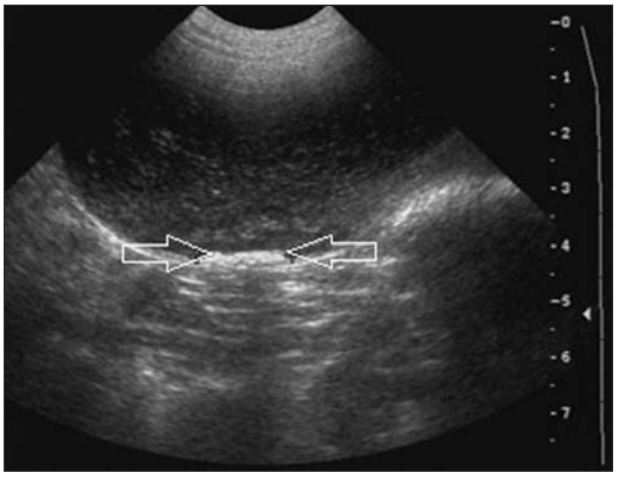

Figure 1—Representative ultrasonographic image of the urinary bladder of a 43-kg (95-lb) 4-year-old neutered male mixed-breed dog evaluated because of dysuria. Notice the hyperechoic urolith (arrows). The diameters of the uroliths in the bladder were ≤ 0.49 cm. Numbers on the right side of the image indicate centimeters.

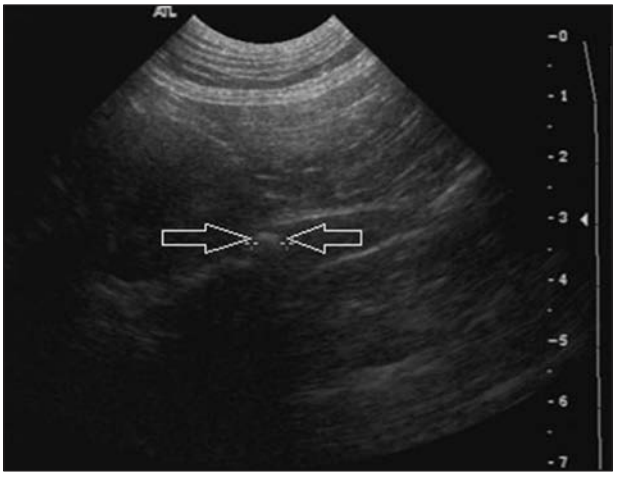

Figure 2—Representative ultrasonographic image of the right kidney of the dog in Figure 1. Notice the urolith (arrows; diameter, 0.8 cm) in the kidney.

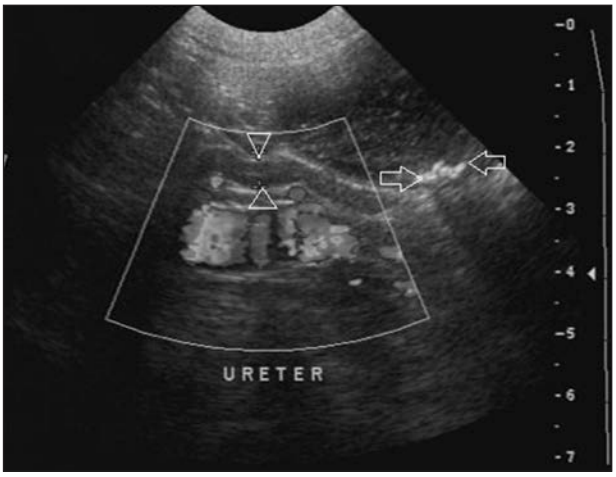

Figure 3—Representative ultrasonographic image of the right ureter (arrowheads) of the dog in Figure 1. Notice the focal region of uroliths (arrows; 1.6 × 1.06 × 2.17 cm) approximately 1.20 cm distal to the right kidney. The ureter is dilated (diameter, 0.56 cm) along the entire length. Renal blood vessels are adjacent to the dilated ureter (indicated by the Doppler ultrasonographic signal).

Figure 4—Photographic image of a urolith surgically removed from the right ureter of the dog in Figure 1. The distance between each line on the scale at the bottom of the image is 1 mm.

tion of renal pelvic dilation and a dilated ureter indicated the dog had an obstruction of the right ureter.

A right ureterotomy was performed via a ventral midline celiotomy in the dog. One large (1.6 × 1.06 cm) slightly brittle green urolith was removed from the right ureter (**Figure 4**). The ureter was closed in 2 layers with 5-0 poliglecaprone 25[d] in a simple interrupted followed by a simple continuous pattern. A cystotomy was performed. Several small uroliths were found in the urinary bladder. A red rubber catheter (10F) was passed in a retrograde direction through the urethra into the bladder, and a large urolith was found. The cystic calculi were removed. The red rubber catheter was passed in a normograde direction from the bladder through the urethra, and no additional uroliths were detected. The cystotomy was closed with 3-0 poliglecaprone 25[d] in a simple continuous pattern. The abdomen was lavaged with sterile saline (0.9% NaCl) solution. The linea alba was closed with size-1 polydioxanone in a simple continuous pattern, the subcutaneous tissues were closed with 2-0 poliglecaprone 25[d] in simple continuous patterns in 2 layers, and skin was closed with surgical skin staples. Postoperatively, the dog received tramadol hydrochloride (2.3 mg/kg, PO, q 8 h) and amoxicillin-clavulanic acid (10.8 mg/kg [4.9 mg/lb], PO, q 12 h) for 14 days.

The uroliths removed during surgery were submitted to a laboratory[e] for analysis. The large urolith from the ureter was dark green, was friable, and had multiple crystallization centers. Results of scanning electron microscopy and energy-dispersive x-ray spectroscopy indicated the urolith contained only carbon, nitrogen, and oxygen; sodium or ammonium urate was not detected (**Figure 5**). Results of Fourier transform infrared spectrometry indicated the urolith contained 2,8-DHA (**Figure 6**); ammonium and sodium urate, xanthine, and uric acid were not detected (**Figure 7**).

Because results of the initial urolith analysis suggested the uroliths contained ammonium urate, serum bile acids concentrations of the dog were measured. Preprandial (5.7 µmol/L; reference interval, < 10 µmol/L) and postprandial (1 µmol/L; reference interval, < 20 µmol/L) serum bile acids concentrations were within the reference intervals.

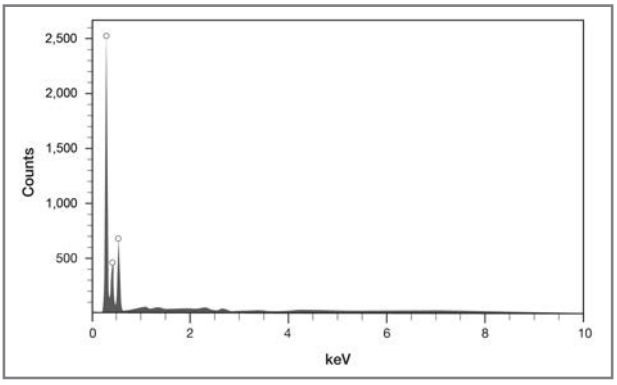

Figure 5—Graph of energy dispersive x-ray spectroscopy data for ureteral uroliths from the dog in Figure 1. Results indicate the urolith contains carbon, nitrogen, and oxygen. No sodium or uric acid salts were detected.

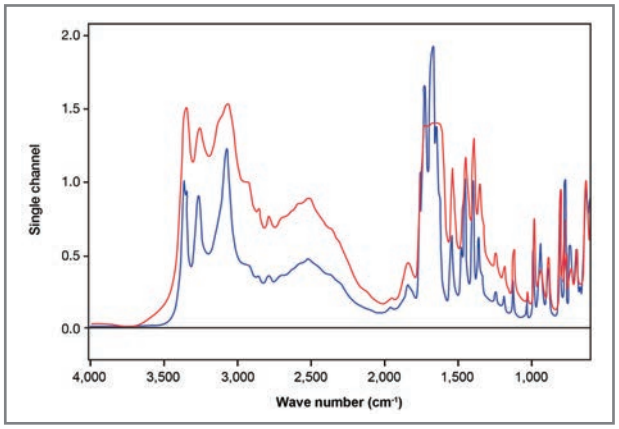

Figure 6—Results of Fourier transform infrared spectrometry of a urolith from the dog in Figure 1 (red line) and a reference substance for 2,8-DHA (blue line).

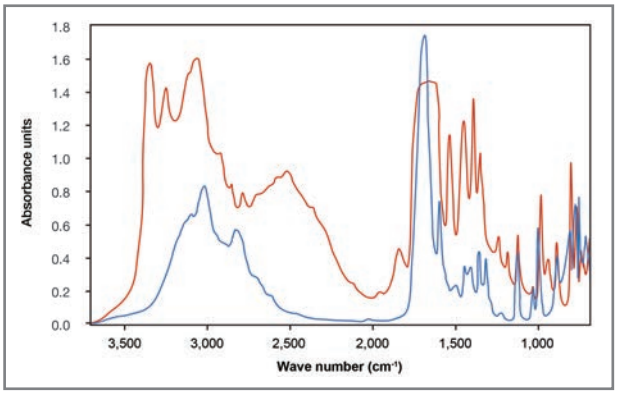

Figure 7—Results of Fourier transform infrared spectrometry of a urolith from the dog in Figure 1 (red line) and a uric acid control sample (blue line).

Results of analyses indicated that the calculi were 2,8-DHA uroliths. To further investigate the cause of 2,8-DHA urolith formation, a blood sample (3 mL) was obtained from a jugular vein of the dog and placed in a tube containing EDTA for determination of RBC APRT and HPRT activities. A free-catch urine sample (5 mL) was obtained from the dog for detection of 2,8-DHA and 2,8-DHA metabolites. The blood and urine samples were shipped at ambient temperature to a laboratory[f] for analysis. At that same referral hospital and during that same day, blood and urine samples were obtained from a control dog and submitted to the laboratory[f] for analysis. The owner of the control dog consented to collection and analysis of the blood and urine samples.

At the laboratory,[f] 1:31 dilutions of urine samples (of the dog that had uroliths and the control dog) were prepared and analyzed by use of an ultraperformance liquid chromatography system with photodiode array detection. The urine sample obtained from the dog that had uroliths contained 0.263 mmol of 2,8-DHA/L. No 2,8-DHA was detected in the urine sample of the control dog. Uric acid concentration in the urine sample of the dog that had uroliths was low (0.258 mmol/L), compared with the uric acid concentration in the urine sample of the control dog (1.421 mmol/L).

Red blood cell APRT and HPRT activities were determined for the dog that had uroliths and the control dog. Red blood cell lysates were prepared: 1:6 dilutions of RBCs (100 µL of packed RBCs in 500 µL of water) were placed in 1.5-mL tubes.[g] Three tubes (duplicate assay samples plus a control sample) of RBC lysates for each dog were prepared for each enzyme (APRT and HPRT) assay. Phosphoribosyl-pyrophosphate (2.5 mmol in 125 mmol of Tris [pH, 7.4]) or hypoxanthine (5mM in Tris [pH, 7.4]) was added to tubes of RBC lysates. Tubes were incubated (15 minutes, 37°C), and reactions were stopped by addition of trichloroacetic acid (40%). Enzyme (APRT and HPRT) activities were measured by use of an ion-pairing, reversed-phase high-performance liquid chromatography system. Red blood cell APRT activities were 10.3 and 8.7 nmol/h/mg of hemoglobin for the dog that had uroliths and the control dog, respectively (reference interval, 16 to 32 nmol/h/mg of hemoglobin). No HPRT activity was detected in RBC lysates for either dog (reference interval, 80 to 130 nmol/h/mg of hemoglobin).

Allopurinol (10 mg/kg [4.5 mg/lb], PO) was prescribed for the dog that had uroliths, and a purine-restricted diet was recommended. Because a renal urolith remained in the dog, the owner was instructed to monitor the dog carefully for signs of upper or lower urinary tract disease. Follow-up recommendations included urinalysis and abdominal ultrasonography every 3 months and routine monitoring of renal function.

The dog was brought to the clinic by its owner 14 months after surgery for evaluation of swelling at the base of the prepuce and inguinal area. Allopurinol had not been administered as recommended. Results of physical examination, serum biochemical analyses, and hematologic analyses were unremarkable; no abnormalities were identified in the prepuce or base of the penis. Results of urinalysis indicated a low urine specific gravity with ammonium urate crystalluria (0 to 5 crystals/hpf). Ultrasonographic findings included loss of corticomedullary definition, mild mineralization in the renal pelvis, and a mildly irregular contour of the right kidney. Small cystic calculi were detected in a 1.55 × 0.33-cm region in the dorsal aspect of the bladder, with individual calculi measuring up to 0.29 cm. Recommendations included feeding of a purine restricted diet[h] and monitoring via urinalysis and abdominal ultrasonography every

3 months for 1 year followed by 2 to 3 times annually; it was considered likely that the dog could pass the small bladder uroliths, and the owners were instructed to monitor for possible obstructive uropathy.

One month later, the dog was clinically normal. At that time, pre- and postprandial bile acids concentrations were 1 and 4 µmol/L (reference ranges, 0 to 6 and 0 to 15 µmol/L), respectively. Administration of allopurinol (10 mg/kg, PO) and performance of another urinalysis in 4 weeks to monitor for xanthine crystalluria were recommended. Performance of ultrasonography, urinalysis, and renal function tests was recommended 3 months later.

Discussion

2,8-dihydroxyadenine uroliths are extremely rare in dogs. A 2,8-DHA urolith was found in a 4-year-old neutered male Schipperke.[1] In that dog, no crystals were found during urinalysis and no urinary tract infection was detected. The urolith in that dog was dark green, was 7 mm long, and had a rough surface. The urolith was surgically removed and determined to be composed of 2,8-DHA via infrared spectrometry.[1] Unfortunately, that dog was euthanized within 1 year after the urolith was removed because of an unrelated problem, and no follow-up information was available. Uroliths composed of 2,8-DHA have been identified in cattle,[2] humans,[3–7] and *Aprt* knockout mice.[8] In humans, 2,8-dihydroxyadeninuria is induced by APRT deficiency resulting from a rare autosomal recessive disorder caused by 1 of 24 genetic mutations involving chromosome 16q24.[9] In humans with APRT deficiency, adenine is oxidized to 2,8-DHA by xanthine oxidase via 8-hydroxyadenine[9]; 2,8-DHA is poorly soluble in urine at any pH and detection of 2,8-DHA in urine is pathognomonic for APRT deficiency in humans.[9,10,15]

Two types of APRT deficiency have been identified in humans. Adenine phosphoribosyltransferase activity is very low or undetectable in lysates of RBCs obtained from humans (typically of Caucasian ancestry) with type 1 APRT deficiency.[9] Type II APRT deficiency is characterized by low residual APRT activity (10% to 25%) in RBC lysates and is identified almost exclusively in humans of Japanese descent.[9]

Humans that are heterozygous for genetic mutations causing APRT deficiency do not have clinical signs of disease.[11,12] Humans that are homozygous for genetic mutations causing APRT deficiency may be asymptomatic but typically develop recurrent nephrolithiasis.[10] Humans with APRT deficiency can have obstructive uropathy, acute renal insufficiency, or progressive chronic renal failure.[12] Adenine phosphoribosyltransferase deficiency is likely in humans that have uric acid uroliths, circulating uric acid concentrations within the reference range, and characteristic round yellow to red-brown urine crystals with dark outlines and central spicules.[9,10] Results of genetic studies indicate > 1% of humans in Japan are heterozygous for mutations in alleles of the gene coding for APRT. On the basis of the variable clinical signs of APRT deficiency and the potential for misdiagnosis of APRT deficiency as uric acid urolithiasis, 2,8-dihydroxyadeninuria is likely underdiagnosed in humans.[4,10,13,15]

In the dog with 2,8-DHA uroliths of the present report, ultrasonography of the urinary system revealed renal, ureteral, and cystic uroliths; these uroliths were radiolucent. Because urate and 2,8-DHA uroliths are both purine-type uroliths, it was not surprising that one laboratory identified the calculi as urate uroliths whereas the other laboratory identified calculi as 2,8-DHA uroliths. It is difficult to distinguish between urate and 2,8-DHA uroliths. Urate and 2,8-DHA uroliths are both radiolucent and have identical chemical reactivities.[9] Urate uroliths are hard, are difficult to break, and have a smooth surface with a faint yellow color; 2,8-DHA uroliths are soft, are friable, and have an irregular surface with a gray or green color.

Spectrophotometric, infrared spectrophotometric, crystallographic, genetic, and enzyme (determination of RBC lysate activities of adenine and hypoxanthine or guanine phosphoribosyltransferases) analyses are used to identify 2,8-DHA uroliths in humans with complete APRT deficiency.[11] In the dog of the present report, serum bile acids concentrations were within the reference intervals, indicating the dog had clinically normal liver function. Results of infrared spectrophotometry indicated the uroliths were more likely composed of 2,8-DHA than uric acid. Results of additional analyses of urine samples indicated the dog had 2,8-dihydroxyadenuria. Complete APRT deficiency was not diagnosed in the dog of the present report. Further studies are warranted to identify 2,8-dihydroxyadenuria in dogs.

To the authors' knowledge, 2,8-DHA uroliths cannot be dissolved in vivo. Administration of allopurinol, a xanthine oxidase inhibitor, is recommended to reduce production of 2,8-DHA in humans.[5] This drug is typically administered concurrent with feeding of a purine-restricted diet to decrease production of xanthine uroliths. In humans, proper preventive measures such as administration of allopurinol and feeding of a purine-restricted diet markedly decrease the risk for recurrence of 2,8-DHA uroliths.[10,14] Allopurinol was prescribed for the dog of the present report, and a purine-restricted diet was recommended.

Because 2,8-DHA uroliths may be underdiagnosed in humans, such uroliths may be underdiagnosed in dogs; analysis of uroliths from dogs to detect 2,8-DHA may be warranted. In particular, uroliths of dogs of breeds that do not frequently have urate urolithiasis and dogs with clinically normal liver function and suspected purine urolithiasis should be analyzed to detect 2,8-DHA.

a. Prescription Diet canine j/d, Hill's Pet Nutrition Inc, Topeka, Kan.
b. Clavaseptin, Vetoquinol Canada Inc, Lavaltrie, QC, Canada.
c. Minnesota Urolith Center, Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Minnesota, Saint Paul, Minn.
d. Monocryl suture, Ethicon Inc, Somerville, NJ.
e. Canadian Veterinary Urolith Centre, Laboratory Services Division, University of Guelph, Guelph, ON, Canada.
f. Purine Research Laboratory, Guy's Hospital, London, England.
g. Safe-lock Tubes, Eppendorf, Oldenburg, Germany.
h. Royal Canin Canine Veterinary Diet Hypoallergenic HP, Guelph, ON, Canada.

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