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Case Report
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Ectopic Bone as a Nidus for Calcium Oxalate Urocystolithiasis in a Cat

J.P. Lulich, H.S. Prasad, M. Manno, and B. Bagley

A 7-year-old female spayed domestic shorthair cat was referred to the urology service for nonsurgical urocystolith removal. A urolith was attached to the urothelium by ectopic bone. Interventional removal without surgery was successful. Follow-up evaluation 3 years after urolith removal revealed recurrent uroliths, bladder wall mineralization, and marked renal mineralization. This case illustrates the metaplastic potential of the urothelium and that ectopic bone should be included among the pathological factors that promote lithogenesis.

Key words: Dystrophic mineralization; Ectopic ossification; Heterotopic bone.

A 7-year-old female spayed domestic shorthair cat was referred to the urology service at the Veterinary Medical Center, University of Minnesota, for nonsurgical urocystolith removal. Three months previously, survey of abdominal radiographs performed to evaluate an acute episode of diarrhea identified 2 irregularly shaped radiopaque densities consistent with uroliths in the body and neck of the urinary bladder. Signs of lower urinary tract disease had not been recognized. The cat was consuming commercially prepared maintenance foods[a] and had not received supplements or medications to maintain health or treat disease for the past 6 months.

On presentation, this 3.3-kg cat was physically normal. Abnormalities were not detected on the serum biochemical profile. Urine obtained by cystocentesis had a specific gravity—1.040, occult blood—3+, 20–50 RBC/HPF, and 0–5 crystals of unidentifiable shape/HPF. Survey abdominal radiographs confirmed that the previously diagnosed uroliths had not been spontaneously voided (Fig 1).

Cystoscopy was performed with the cat anesthetized and in dorsal recumbency. The vestibule of the vagina and the urethra was visibly normal. Upon entering the urinary bladder, 2 apparent uroliths were identified (Video S1). One was embedded in the ventral bladder wall (Fig 2). It was retrieved with a stone-extracting basket,[b] inserted through the biopsy channel of the cystoscope.[c] Once grasped and pulled, the apparent urolith was easily freed from its attachment (Video S2). Because it was small enough to pass through the urethra, the urolith was retrieved intact. Although its macroscopic appearance was originally consistent with a urolith, close inspection revealed a firm soft tissue base at its attachment to the bladder wall. The remaining urolith within the body of the urinary bladder was freely movable and too large to be transported through the urethra; it was fragmented with holmium–YAG laser technology and removed in sections. The cat recovered uneventfully.

Abbreviations:

BMP	bone morphogenic protein
CaOx	calcium oxalate
CaP	calcium phosphate apatite
CT	computerized tomography
EDAX	energy-dispersive analysis of X-rays
FTIR	Fourier transform infrared spectroscopy
HPF	high-power field (×450)
SEM	scanning electron microscopy

The attached urolith was evaluated by microcomputerized tomography (CT) to determine the orientation of its exterior and interior anatomy (Video S3 and S4). Then, it was stored in 10% neutral-buffered formalin. After dehydration through a graded series of alcohols, the urolith was infiltrated with a light-curing embedding resin.[d] Sections of approximately 50 μm thick were prepared from a region that bisected the urolith in half. Sections were mounted on glass slides and stained with Stevenel's blue and Van Gieson's picro fuchsin (Fig 3). Microscopic evaluation showed a urolith base histologically consistent with bone; it retained stain and possessed lacunae and lamellae. The outer mineral portion of the urolith did not retain stain and consisted of organized radiating plates with unorganized granular crystals in the middle section. Reflectance micro-Fourier transform infrared spectroscopy (FTIR) was performed on unstained sections. The major chemical composition of the base was identified as calcium phosphate apatite (CaP), and the major

From the Veterinary Clinical Sciences, College of Veterinary Medicine, (Lulich); Oral Pathology, College of Dentistry, University of Minnesota, (Prasad); Chemical Engineering and Materials Science, College of Science and Engineering University of Minnesota, (Manno); Earth Sciences, College of Science and Engineering, University of Minnesota, Minneapolis, MN (Bagley).

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Corresponding author: J. Lulich, Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota, 1352 Boyd Ave, St Paul, MN 55108; e-mail: lulic001@umn.edu

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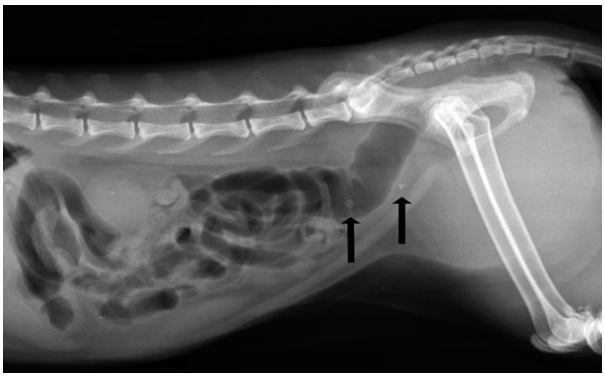

Fig. 1. Lateral abdominal radiograph of a 7-year-old female spayed domestic shorthair cat showing 2 radiopaque uroliths in the plane of the urinary bladder (arrows). A small density over the plane of the left kidney is consistent with a nephrolith.

composition of the overlying mineral was identified as calcium oxalate (CaOx) monohydrate. A 50-nm-thick carbon layer was sputtered over the surface of the bisected section of the sample in preparation for scanning electron microscopy (SEM). Backscatter electron imaging (Fig 4) revealed that the base region of the urolith (noted with the asterisk symbol) contained morphological structures consistent with lacuna and lamellae. Furthermore, the base region produced a higher yield of backscattered electrons (i.e., "brighter region") compared with the mineral portion of the urolith (noted with the hash symbol) signifying that the base region was of higher mass density and average atomic number. Energy-dispersive analysis of X-rays (EDAX) across the base to the mineral boundary (Fig S1) revealed an abrupt decrease in the phosphorus to calcium (P/Ca) atomic ratio from ~ 0.66 to ~ 0.08 when scanning from the base region to the mineral region. These observations were consistent with the micro-FTIR analysis that the base was composed of CaP with the overlaying mineral composed of CaOx monohydrate. The unattached urolith was composed of 100% CaOx monohydrate.

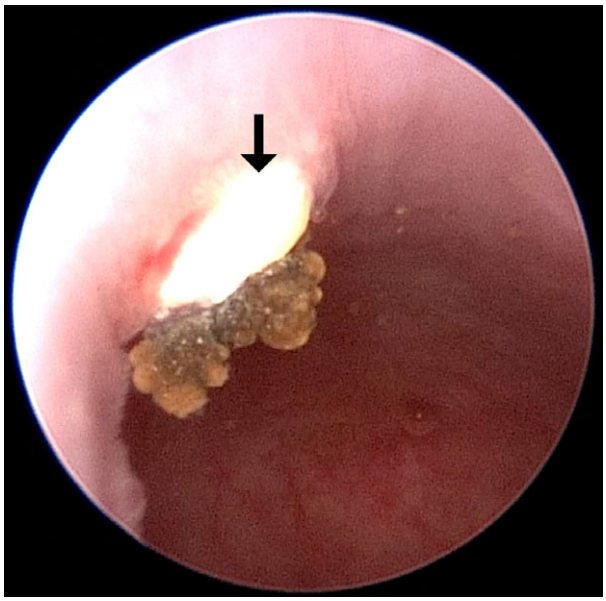

Fig. 2. Cystoscopic image from a 7-year-old female spayed domestic shorthair cat with an apparent urolith adhered to the mucosa of the ventral right neck of the urinary bladder. The attachment (arrow) was bone (see Fig 3), and the outer portion was composed of calcium oxalate monohydrate.

Three years after urolith removal, medical imaging (survey abdominal radiography and ultrasonography) revealed a recurrence of a mineral focus in the caudal aspect of the urinary bladder consistent with an adherent urolith or tissue mineralization (Fig S2). A small urolith within the bladder lumen was also identified (Fig S3). Ultrasound examination of the kidneys revealed marked parenchymal mineralization (Fig 5). The length of the left kidney which measured 2.4 cm previously was now 1.9 cm. The cat had no clinical signs. Abnormalities were not detected on the serum biochemical profile (creatinine = 1.8 mg/dL; normal is 0.5–2.1; phosphorus = 3.3 mg/dL; normal = 3.3–7.8, and

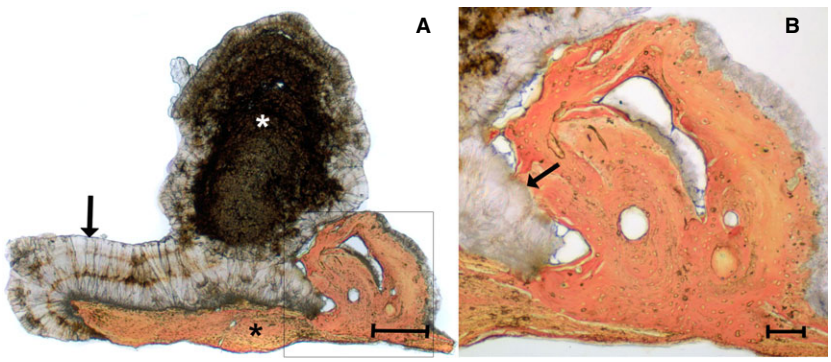

Fig. 3. Undecalcified histopathological section through the middle of the urolith and stained with Stevenel's blue and Van Gieson's picro fuchsin (panel **A**). The base of the urolith stained red consistent with collagen of bone (black asterisk). The mineral portion of the urolith was not vital and therefore did not stain. The overlying mineral was composed of organized radiating plates of calcium oxalate monohydrate (arrow). The middle portion consisted of unorganized granular calcium oxalate monohydrate (white asterisk; bar = 500 μm). Panel **B** is a higher magnification of the square insert outlined in panel **A** and shows thick trabecular bone, bone lacunae without nuclei, and the close interdigitation (arrow) between bone and covering mineral (bar = 250 μm).

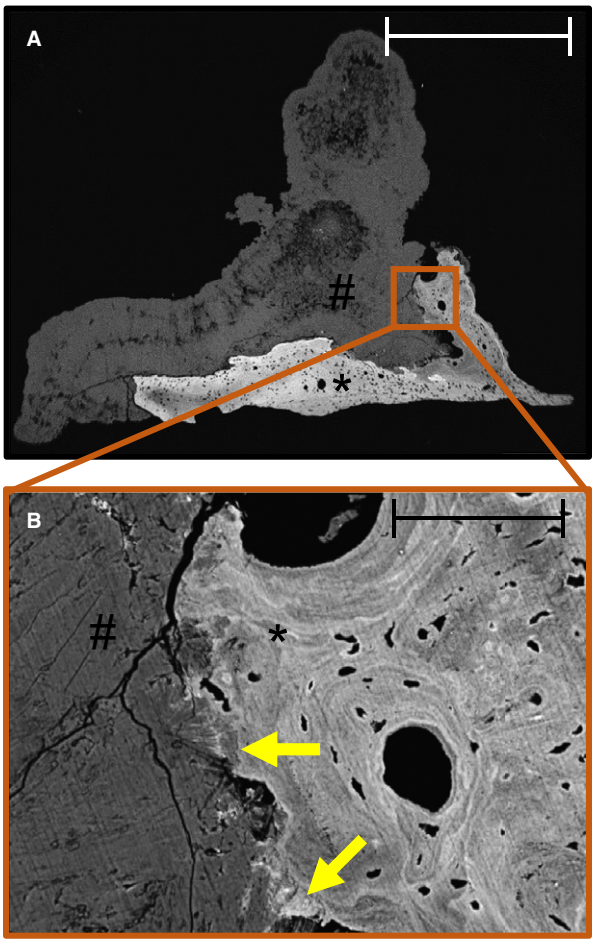

Fig. 4. Scanning electron microscopy backscatter electron image of bisected urolith (panel **A**). The base of the urolith (asterisk) produced higher amounts of backscatter electron yield compared to the overlying mineral region (hash symbol), indicating that the base of the urolith consisted of elements that had an overall higher atomic number or mass density (bar = 1 mm). Panel **B** is a close-up view of the insert in panel **A**. Lacunae and lamella in bone are readily visible as well as its interdigitation (arrows) with the mineral region (bar = 100 μm).

total calcium = 10.2 mg/dL; normal = 8.3–10.9). Serum concentrations of ionized calcium[e] (1.3 nmol/L; normal = 1.0–1.4), and PTH[e] (0.9 pmol/L; normal = 0.4–2.5 pmol/L) were within reference ranges for normal cats. Urine obtained by cystocentesis had a specific gravity—1.013, occult blood—1+, occasional RBC/HPF, and no crystals. During the interval between urolith removal and follow-up evaluation, treatment designed to prevent urolith recurrence was not initiated. Instead, the cat was fed a therapeutic food[f] for intestinal disease along with its previous diet.[a]

Discussion

Ectopic bone, also called heterotopic ossification, is a disease condition describing the formation of histological bone in locations where osseous tissue is not normally found.[1] Ectopic bone is not related to pathological calcification, a lesion in which calcium salts deposit in dead or dystrophic soft tissues, or because of increases in serum calcium concentration. However, there might be overlap in these 2 disease processes.

The pathophysiology of ectopic bone formation is not well understood but excessive bone morphogenic protein (BMP) signaling in an inflammatory environment together with increased expression of other osteogenic cytokines results in heterotopic ossification in animal models.[2,3] The mechanisms underlying ectopic bone in cats are not known. However, a supply of mesenchymal cells whose genetic makeup is not fully committed would be a requirement. Once given an appropriate signal, uncommitted mesenchymal cells differentiate into osteoblasts that promote deposition of bone.[4]

Ectopic ossification originating from the urothelium of the left renal pelvis had been reported in a 3-year-old cat with left hydronephrosis.[5] This case report also illustrated that ectopic bone can be easily dislodged from the urothelium without surgical extirpation.

From the data in this cat, it was not possible to postulate which factors increased the likelihood of ectopic bone. In other species, trauma followed by inflammation and exuberant repair is often identified as the inciting process.[6,7] The cat in our case report did not have previous urinary tract surgery, or clinical signs or clinicopathologic findings indicative of previous urinary tract disease. Although cystocentesis is routinely performed to obtain urine for analysis in cats and the

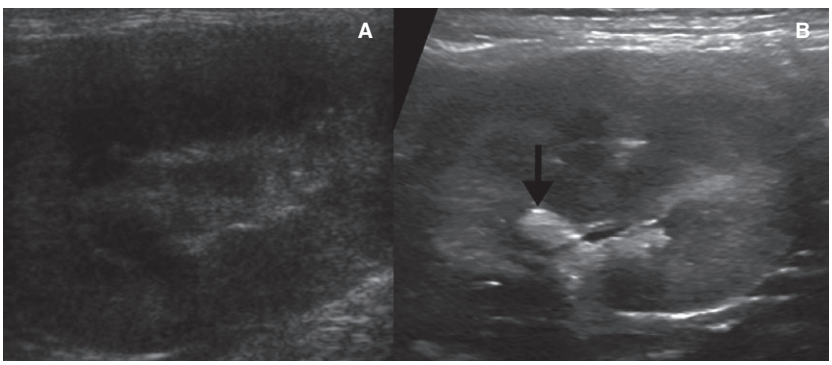

Fig. 5. Ultrasound images of the right kidney of the cat depicted in Fig 1 at the time of urolith removal (panel **A**) and 3 years later (panel **B**). Several hyperechoic and shadowing foci consistent with mineral are throughout the renal cortices and pelvic tissues (arrow) 3 years after urolith removal.

procedure can potentially induce urothelial trauma, the location of the ectopic bone was caudal to the typical site of needle insertion. However, this cat had a larger urolith in the bladder lumen. Although a urolith can be a potential source of trauma, it was impossible to determine which came first, the larger urolith or the bone. In several case series and case reports of human nephroliths in which bone was identified, bone was often located in the center of uroliths, a finding consistent with mineral precipitating over pre-existing bone.[8–10] It is possible that given time, a similar urolith appearance would have occurred in this cat. Therefore, we hypothesize that bone formed first and CaOx mineral precipitated over it.

Questions still remain about the management and potential reformation of ectopic bone and uroliths. Three years after initial urolith removal, a recurrent urocystolith and bladder neck mineralization were detected in this cat. Surgical removal of ectopic bone would have removed abnormal tissue; however, if trauma is a potential risk factor, surgery could have accelerated disease. Nonsteroidal anti-inflammatory medication and radiotherapy have delayed ectopic bone formation after hip replacement surgery in humans and could represent potential therapeutic strategies for cats.[1] Although not performed, renal biopsy in this cat would have helped determine whether progressive mineralization of the kidneys was related to ectopic bone in the bladder. As this cat had 3 mineral-related diseases, ectopic bone, CaOx uroliths, and renal mineralization, we hypothesize an etiopathological link between them. This assumption is plausible as known causes for tissue mineralization, such as azotemia and hypercalcemia, were not detected in this cat.

The clinical significance of this case is that ectopic ossification should be included among the disease factors that promote lithogenesis. Of particular interest is the mechanism of CaOx deposition over bone. Therapeutic strategies would differ if urolith formation is a function of supersaturated urine in contact with a preexisting nidus, the bone; or if local factors, such as osteogenic mediators at the site of ectopic bone, recruit and precipitate calcium salts, in which treatment to reduce supersaturation may benefit minimally.

Footnotes

[a] Fancey Feast cat food; Nestle Purina PetCare Company, St Louis, MO.
[b] NCircle Nitinol Tipless Stone Extractor; COOK Medical, Bloomington, IN
[c] Mini multi-purpose rigid telescope; Karl Storz Endoscopy Inc., El Segundo, CA
[d] Technovit 7200VLC; Kulzer, Wehrheim, Germany
[e] Veterinary Diagnostic Laboratory, Michigan State University, Lansing, MI
[f] Prescription diet feline i/d; Hills Pet Nutrition, Topeka, Kansas

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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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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Scanning electron microscopy secondary electron image of bisected urolith across the base – mineral boundary (panel A).

Figure S2. Lateral abdominal radiograph of the cat depicted in Fig 1.

Figure S3. Ultrasound images of the urinary bladder of the cat depicted in Fig 1 at the time of urolith removal (panel A) and 3 years later (panel B). Panel B shows a small recurrent urocystolith. The mineralized area in the bladder neck was not identified at either the initial or later ultrasound evaluation.

Video S1. Cystoscopy in a 7-year-old DSH cat revealed a movable urolith free within the urinary bladder and a urolith attached to the ventral wall of the caudal bladder neck.

Video S2. Cystoscopy in a 7-year-old DSH cat showed how the urolith attached to the ventral wall of the caudal urinary bladder was dislodged and removed using a stone retrieving basket.

Video S3. Micro-computerized tomography of a urolith retrieved from the urinary bladder neck of a 7-year-old DSH cat. The urolith is rotated to evaluate the external surface.

Video S4. Micro-computerized tomography of a urolith retrieved from the urinary bladder neck of a 7-year-old DSH cat. The urolith is gradually bisected to explore the internal structure. The base of the urolith was composed of bone. The material above the bone was calcium oxalate monohydrate.