Original Article

Surgical Removal of Urinary Bladder Urolith in Female Asian Elephant (*Elephas maximus*) by Episiotomy and Urinary Bladder Sphincterotomy

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Abstract

A 50-year-old female Asian elephant (*Elephas maximus*) was referred to the Kasetsart University Veterinary Teaching Hospital with histories of anuria and abdominal pain for five days. Trans-rectal ultrasonography demonstrated the presence of hyperechogenic uroliths located between the neck and trigone of the urinary bladder. Vestibuloscopy by endoscope revealed an abnormal brownish mass obstructing the internal bladder orifice. Episiotomy was performed after standing sedation with dexmedetomidine, epidural anesthesia and perineal infiltration with lidocaine. A single 1.7 kg urinary bladder urolith with 17x12x10 cm dimension was manually removed using a combination of several methods, including sphincter dilatation using lidocaine ring infiltration at the sphincter, blind sphincterotomy, rope woven basket and intraluminal inflation with gas. The episiotomy site was left open as a window for further wound cleaning and urinary bladder lavage. Signs of chronic kidney disease were detected and post-operative care was started. However, this elephant died on day 80 after the surgery. A necropsy was performed and the major finding was severe diffuse necrosis of both kidneys. Calcium carbonate was identified as the urolith's main component. Predisposing factors for urolith formation in this case remains unclear.

Keywords: Asian elephant, episiotomy, sphincterotomy, urinary bladder, urolithiasis

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Introduction

A combination of alkalinity and a high concentration of calcium excreted in elephant urine (Wiedner, 2015; Wiedner *et al*., 2009; Miller, 2006) creates a good environment for urolith formation. However, urolithiasis is infrequently diagnosed in elephants and usually accidentally found during necropsy or urogenital examination. Two calcium carbonate stones were found in the left ureter during necropsy of an African elephant (*Loxodonta africana*) that died from chronic renal failure (Morris *et al*., 1987). Later, in 1995 urinary stones were identified during urethral endoscopic examination in a male Asian elephant (Ruedi, 1995). Reports on treatment of urolithiasis in elephants are also rare. There has been only one report of urethrotomy for stone removal in a male Asian elephant, followed by a permanent urethral fistula as a complication after surgical treatment (Hildebrandt *et al*., 2000; Lange *et al*., 1999). Recently, episiotomy and urethrotomy were performed to remove 162 small urethral calculi with a total weight of 8 kg in a female Asian elephant (*Elephas maximus*) (Thongtip *et al*., 2013). After the surgery, urethral rupture and secondary wound healing were noted during post-operative care in this elephant (Thongtip *et al*., 2016).

In this case report, a 1.7 kg of bladder calculus 17x12x10 cm in dimension was surgically removed from the urinary bladder (UB) of a female Asian elephant (*Elephas maximus*). Details in this report include diagnosis, surgical procedure via episiotomy, bladder sphincter dilatation with local anesthesia and sphincterotomy and postoperative care are described.

Animal signalment and clinical signs: A 3,200 kg 50-year-old female Asian elephant with clinical signs of restlessness (sitting down and rising up repeatedly), abdominal pain and anuria for four days (first noticed by a mahout on 25 March 2021) was referred to the Veterinary Teaching Hospital of Kasetsart University, Kamphaeng Saen Campus on 29 March 2021. Upon arrival, the clinical sign of repeated sitting down and rising up was no longer present but clinical signs of anuria were still present. The elephant also showed signs of depression, loss of appetite and no defecation. Vital signs including body temperature, pulse rate and respiratory rate were 99^0 F, 35 beats per minute and 5 times per minute, respectively. The elephant also showed clinical signs of urinary incontinence and turbid urine over the previous six months.

***Laboratory Tests*:** Blood was collected from the auricular vein for pre-operative hematology and serum chemistry examinations. For urinalysis, the midstream urine was collected using a 50 ml centrifuge tube during perioperative on the operation day, day 5 and day 17 after the operation. Results of hematology and serum chemistry are shown in Table 1. Results of urinalysis are shown in Table 2.

Table 1 Results from hematology and serum chemistry examination one day before surgery (D-1), surgery day (D 0), and 1, 5, 9, 17, 22, 25, 33 days (D 1, D5, D9, D17, D22, D25, D33) after surgery on this elephant.

Parameters	D -1	D 0	D 1st	D 5th	D 9th	D 17th	D 22nd	D 25th	D 33rd	Reference value (Silva & Kuruwita 1993[a,b])
Hct (%)	29.1	28.2	34.1	30.7	28.4	27.3	20.4	20.1	17.9	30-40
Hb (mg%)	10.6	10.8	12.8	11.3	10.6	10	7.3	7.1	6.5	11-15
RBC (x 10^6/µl)	2.55	2.58	3.05	2.73	2.54	2.41	1.82	1.76	1.56	2.5-5.0
WBC (x 103/µl)	9.91	10.39	10.74	15.29	23.05	20.07	17.99	18.14	17.88	10-18
Neutrophil (%)	45	61	64	44	67	47	67	61	65	20-40
Lymph (%)	46	17	21	15	15	18	4	15	24	50-80
Mono (%)	7	17	21	38	18	34	29	18	18	20-40
Eos (%)	2	5	5	2	0	1	0	1	3	1-10
nRBC	0	0	0	0	0	0	0	4	2	NA
Platelet (x 10^5 g%)	238	267	246	243	330	458	424	362	219	200-600
PP (mg%)	6.4	6.4	7.4	7.2	7.6	7.4	7	6.6	7.2	6-12
Total Prot. (g/dL)	5.6	5.2	6.4	6	6.4	6.3	5.5	5.5	5.7	NA
Albumin (g/dL)	1.6	1.5	1.7	1.7	1.6	1.6	1.4	1.4	1.4	NA
Creatinine (mg%)	6.21	7.29	7.67	5.95	3.01	2.17	4.77	4.24	4.82	1.0-2.0
BUN (mg%)	18	16.8	18.2	18	NA	8.3	23.6	NA	NA	5-20
AST (U/L)	24	NA	NA	NA	NA	29	NA	NA	37	15-35

Hb: hemoglobin, Hct: hematocrit, PLT: platelet, PP: plasma protein, nRBC: nucleated red blood cell, Total Prot.: Total protein, BUN: blood urea nitrogen, AST: Aspartate aminotranferase, NA: Not available

Table 2 Result from urinalysis examination on surgery day (D 0), day one and seventeen (D1 and D17) after surgery on this elephant.

Parameters	D 0 (operation)	D 5th	D 17th
Color	Brown	Yellow	Colorless
Transparency	Cloudy	Sediment	Clear
RBC	>20	>20	>20
WBC	0-5	>20	Negative
pH	8	8	8
Sp. Gr.	1.016	1.01	1.008
Protein	+1	+1	+1
Bilinogen	Negative	Negative	Negative
Blood	+4	+4	+4
Ketone	Negative	Negative	Negative
Biririubin	Negative	Negative	Negative
Glucose	Negative	Negative	Negative
Leucocyte	+1	+2	Negative
Bacteria	+3	+4	+1
Fat droplet	Negative	Negative	Negative
Crystal	Calcium oxalate monohydrate, Calcium oxalate dihydrate	Calcium oxalate monohydrate	Calcium oxalate monohydrate
Epithelium	Transitional (0-5), caudal (0-5)	Transitional (0-5)	Negative

***Ultrasonography*:** Transrectal ultrasonography (Mindray Z5 Vet Diagnostic Ultrasound System, SHENZHEN MINDRAY Bio-Medical Electronics Co., LTD., Shenzhen, P.R. China with convex transducer probe; 3.5 MHz) was performed on the arrival date. An abnormal hyperechoic mass with an estimated dimension of 15x10x8 cm was found at the neck of the UB. This mass possessed a hyper echogenicity appearance (Fig. 1) similar to that of the uroliths in the previous case of urolithiasis in a female elephant (Thongtip *et al*., 2013).

Figure 1 The trans-rectal ultrasound over the neck of the urinary bladder reveals a semicircular hyperechoic non-smooth line (large arrow) with intense acoustic shadow (small arrow). This finding suggested that this semicircular hyperechoic non-smooth line is a part of calculi that possesses a rough surface.

***Vestibuloendoscopy*:** Vestibuloendoscopy was performed in a standing position without sedation before the operation using a video endoscope (TELE PACK VET and LED, KARL STORZ SE and Co. KG, Tuttliken, Germany). Routine endoscope technique was not suitable to reach the urogenital junction because of a limitation in visibility inside the vestibule due partly to the large space and collapse of the internal wall of the vestibule. Therefore, inflation of the vestibule with oxygen via the working channel of the endoscope was implemented to expand the vestibular lumen, enhance visibility and expand the urogenital junction. This allowed maneuver of the endoscope into the vestibular opening of the urethra until the tip of the endoscope reached the internal opening of the UB. The internal opening of the UB revealed a light-brown urolith with a rough surface completely blocking urine flow at the neck of the UB. There was no evidence of inflammation or infection in the vestibule.

***Clinical diagnosis and differential diagnosis*:** Clinical diagnosis of this case was based on the history of illness, physical examination, hematology and serum chemistry analysis, ultrasonography and vestibuloendoscopy. Differential diagnosis for this elephant was a bilateral ureteric stone, bladder stone, intraurethral stone, rupture of the UB and acute kidney failure. Based on ultrasonography and vestibuloendoscopy, the final diagnosis of bladder stone was reached.

***Treatment plans*:** The surgical removal of multiple small intraurethral stones in a female Asian elephant via episiotomy over the vestibule and urethrotomy has been reported (Thongtip *et al*., 2013). However, the stone in this case was located inside the UB. The complete obstruction leading to anuria suggested that the stone was bigger than the opening of the sphincter of the UB when relaxed. Episiotomy to explore the urethral canal and the UB was chosen and the techniques that could dilate the sphincter for pulling the stone out were performed. After finishing this operation, the temporary closing of the vestibular wall and skin was also performed. The vestibular wall and skin were sutured together and tension over the wound edge was reduced by applying a full thickness horizontal mattress suture with buttons and rubber mat on both sides of the incision. The incision line was temporally closed with wire that could be untied to open the wound for further cleaning inside the UB during post-operative care.

***Sedation, pre-operative and surgical procedure*:** Prior to sedation, an NSS intravenous fluid therapy was administered at the rate of 3.75 liters per hours for 8 hours through the ear vein. The elephant was sedated by administering 0.0012 mg/kg of Dexmedetomidine hydrochloride (Dexdomitor®, Virbac Thailand Co., Ltd., Nonthaburi, Thailand) intramuscularly. After 20 minutes, the elephant showed signs of sedation including salivation, relaxation of the trunk with the trunk tip resting on the ground and snoring. After adequate sedation, the tail was immobilized with epidural anesthesia using 50 ml of 2% lidocaine hydrochloride (UNION DRUG LABORATORIES LTD, Bangkok, Thailand). Ten minutes after the injection, the tail was successfully immobilized. A line of 20 infiltration sites parallel to the ground and located 5 cm distal to the ventral anal fold were pre-marked with gentian violet. Ten sites were marked on each side of the body. Each site was 3-4 cm apart. To desensitize the skin over the surgical area, 5 ml of 2% lidocaine hydrochloride (UNION DRUG LABORATORIES LTD, Bangkok, Thailand) was infiltrated subcutaneously at the pre-marked sites. The total volume of infiltration was 100 ml. After 15 minutes, the sensation of the skin below the infiltration line was tested and found to be completely desensitized. Prior to an incision, a one-liter intravenous NSS fluid bottle (Siam Medical Care and Supply Co., LtD, Bangkok, Thailand) fully inflated with air in a rectal examination glove was inserted into the vestibule. The NSS bottle was manually stabilized under the median raphe of the vestibule at the proposed incision site. This NSS bottle and manual stabilization helped to stabilize adjacent tissues during the incision which was crucial for creating a full thickness incision, and helped prevent an inadvertent incision of the mucosa of the vestibule on the other side. After the bottle was adequately stabilized, a 25 cm vertical incision line was made over the median raphe of the vestibule 20 cm distal to the ventral fold of the anus. To reduce the tangential shearing force to the subcutaneous tissue between the skin and mucosa of the vestibule, the skin around the wound edge was cramped with the mucosa of the vestibule three to four inches apart using 15 cm surgical towel cramps. The urethral canal and the UB sphincter were explored by hand covered with a rectal examination glove. The examination revealed that the urethral sphincter was approximately 5 cm in diameter (could be inserted with three-four fingers) and the stone was pushing against the internal opening of the UB and completely blocked the urine flow. During the initial manual exploration, the stone was pushed forward and dislodged from the obstruction site which immediately emptied the UB. The urine was serosanguineous in color. The total volume of the urine could not be collected for measurement due to the sudden expulsion but enough was collected for urinalysis.

After the bladder was emptied, the stone dropped into the UB lumen and could be partially grasped with the fingers but could not be removed through the urethral sphincter. Based on the previous ultrasonographic examination and manual exploration, the diameter of the stone was known to be more than 15 cm and it seemed impossible to remove it by hand through the 5 cm diameter urethral sphincter. Therefore, several techniques were implemented to dilate the sphincter. At first, a blind circular injection of 2% lidocaine into the sphincter muscle was performed by using a 1.5-inch 21 G hypodermic needle connected to the infusion line and extension cord. The sharp point of the needle was covered by pinching it between the thumb and index finger. The needle was advanced into the urethral canal and 5 ml of 2% lidocaine was injected into the periphery of urethral canal at multiple locations in the narrowest area. This method was repeated three times at different sites. Ten minutes after the last injection, the urethral sphincter was completely dilated (approximately 10 cm in diameter), as indicated by a lack of sphincter tone and the size of the urethral canal was reached by fully stretching the urethral mucosa. Attempts to remove the stone by hand were repeated several times but still without success. Therefore, a blind sphincterotomy at six locations around the urethral canal was implemented. To perform the sphincterotomy, the sharp edge of surgical blade without a blade holder was covered in a fist and advanced into the urethral canal. The internal wall of the urethra sphincter at the neck of bladder was longitudinally incised in approximately five to ten cm at six locations (2, 4, 6, 8, 10 and 12 o'clock). After sphincterotomy, the urethral canal was large enough for the fingers to pull the stone into the proximal part of the urethral canal but still could not be removed. This was due partly to the rough surface of the stone pushing against the connective tissues around the urethral canal. Several methods were tried to remove the stone during this period. The first method was to saw the stone into small pieces using a fetotome set. This was not successful because the stone could not be held in a fetotome wire. The second method was placing the stone into a plastic bag to reduce the friction between the surface of the stone and connective tissue around the urethral canal. This also was not successful because of an inability to put the stone into the bag. The third method was placing the stone into a hand-woven basket made from plastic rope (Fig. 2) and pulling on the rope. The surgeon was able to place the stone into the basket but the rope connected to the basket broke during several attempts. During this period, if the stone was released, it felt back into the bladder lumen.

Based on these limitations, it was decided to inflate the UB with air to increase the intraluminal pressure of the UB that might help in holding the stone in the urethra canal during stone maneuver. After the stone was successfully placed into the hand-woven basket, an air tube was catheterized into the lumen of the UB. To remove the stone, a rope connected to the basket was pulled into the urethra and the UB was inflated with oxygen. The inflated bladder was able to secure the stone inside the urethral canal. At this time, the rate of inflation of oxygen was reduced, the rope connected to the basket was gently pulled and the tangential between the surface of the stone and the connective tissues and mucosa was gradually separated by the surgeon's fingers. During these manipulations, the surgeon reported that the stone was gradually moved caudally before it was forcefully expelled by positive pressure inside the UB. The stone weighed 1.7 kg and

was 17x12x10 cm in dimension (Fig. 3). An intra urinary bladder endoscopy was repeated to examine the UB and to ensure that all of the stone was removed. During the operation, urine was constantly dripping through the incision.

Figure 2 Calculus from this elephant in a hand-woven basket made from 3 mm diameter nylon ropes that was used to retrieve the calculus from the urinary bladder.

Figure 3 A 1.7 kg of single calculi (17x12x10 cm in dimension) was removed from the urinary bladder of this elephant.

After the stone was removed, the episiotomy site was left open as a window for further wound cleaning and lavage of the UB. Skin at the edge of the wound around the window was sutured to the internal mucosa of the vestibule with Nylon. Three interrupted full thickness horizontal mattress tension sutures with internal and external button supports were placed on the skin and vestibular mucosa adjacent to the wound on each side of the wound. The support buttons were made from a rubber mat (Fig.5). Material for tension suture was the infusion line. The episiotomy window was temporally closed with the addition of wire tied between the external buttons of each side for further post-operative wound care that required opening of the window. The total operation including the initial sedation took 7 hours and 20 minutes. During the operation, the respiratory rate ranged from 6-8 times per minute and the pulse rate ranged from 32-42 beats per minute.

After the operation, 0.012 mg/kg atipamezole hydrochloride (Antisedan® Zoetis Ltd., Bangkok, Thailand) was administered intramuscularly as an antidote. After the administration, the elephant was fully awake within 30 minutes. During urination, the urine flowed through the vestibular window and did not accumulate in the subcutaneous tissue of the vestibule. The mahout also reported that the appetite of the elephant returned during the night of the operation day.

***Post-operative care*:** The bladder was daily lavaged with 10 liters of sterile NSS using an equine endotracheal tube. Nitrofurazone ointment (Bactacin®, Osoth Inter Lab, Bangkok, Thailand) was applied over the edge of the wound. To prevent bacterial infection, 2.5 mg/day of enrofloxacin (Syvaquinol 100®, Syva Ltd., Leon, Spain) was administered intravenously during the first 10 days. To control pain and inflammation, 1.1 mg/kg of Flunixin meglumine (Neuxyn 5%®, Syva Ltd., Leon, Spain) was administered intravenously once a day during the first 10 days. To prevent gastric ulcers, 2 mg/kg of cimetidine (CIMEG T.P.®, T.P. Drug Laboratories Co. Ltd., Bangkok, Thailand) was administered intravenously once a day. Based on the result of a drug sensitivity test, the antibiotic was changed to 5 mg/kg of Amoxycillin clavulanic acid (AMK®, North China Pharmaceutical Co. Ltd., Hebei, China) for 3 weeks. The injectable supplement contained butaphosphan and cyanocobalamin (Catosal®, Bayer Health Care, Kansas, USA) at a total of 50 ml and was intravenously administered every 2 days during a period of 30 days. Twenty tablets of vitamin B complex and ferrous were also given orally once a day for 30 days. After the operation, the elephant possessed a good appetite and defecated normally. During hospitalization, the elephant slept in a standing position using its head and trunk to support its body weight.

Laboratory tests before the operation and during post-operative care: Blood and serum samples were collected one day prior to surgery (Day -1) for routine hematology and serum chemistry examinations. Subsequently, blood and serum samples were collected at days 1, 5, 9, 17, 22, 25 and 33 after the

operation for post-operative monitoring. Results from hematology showed leukocytosis and neutrophilia on days 5- 33. Evidence of decline in Pack Cell Volume (PCV) was first detected on day 22. The PCV level continued to decline from 20.4 (day 22) to 17.9 (day 33). Plasma protein and total protein were low before and after the operation; the albumin level was very low all the time. The AST levels were within the normal limits in all samples during the hospitalization. The creatinine level was high (6) before the operation and remained high (7) until Day 9. The level decreased to 3.01 and 2.17 in Day 9 and Day 17. However, the level increased again during day 22 and 33.

Urinalysis of the mid-stream urine samples was done on the operation day, day 5 and day 17 after the operation using urine strip tests. The urine sample from day 5 was slightly red and turbid. The urinalysis showed alkalinuria (pH 8), hemoglobinuria and pyuria. Calcium oxalate monohydrate and calcium oxalate dihydrate crystals were also found in the urine sediment. The urine sample from day 17 was transparent with the lowest bacteria level and no WBC when compared to previous results. Urine specific gravity (USG) from day 5 and 17 were isosthenuria (Table 2).

Endoscopic examination after removal of urolith: Endoscopic examinations of the internal wall of the UB and wound from sphincterotomy of the urethral canal were conducted using an endoscope. The first, second, third and fourth examinations were performed on days 10, 20, 29 and 34 after the surgery. Results from the first endoscopic examination found mild to moderate cystitis and the presence of necrotic tissue around the periphery of the UB sphincter. Results from the second and third endoscopic examination found large amounts of necrotic tissue inside the UB in conjunction with a significant decrease in space of the internal lumen of the UB (Fig. 4). Results from the fourth endoscopic examination found significant narrowing of the internal lumen of the UB, enlargement of the right ureteric opening, and narrowing of the left ureteric opening. Further examination of the right ureter found enlargement of the right ureter and the presence of a small amount of tissue debris along of the internal wall of the right ureter. The left ureteric opening was too narrow to advance the endoscope into the left ureter for further examination.

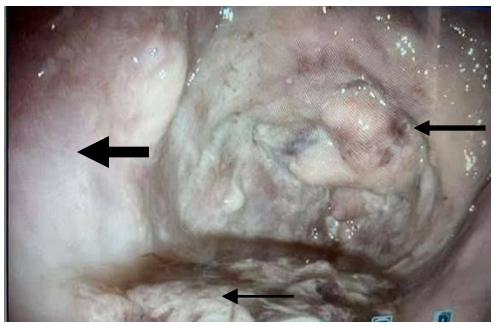

Figure 4 Endoscopic picture inside the urinary bladder 29 day after surgery. Large arrow indicates that the wall shows signs of good healing (light pink in color and not covered by necrotic tissue layer). Medium arrow indicates area of dark pink color suggesting that the wall in these areas were more damaged earlier and now also show sign of good healing. Small arrow indicates that the wall is still covered with a layer of necrotic tissue.

Figure 5 The episiotomy window was temporally closed with the addition of wire tied between the external buttons (red arrow) of each side for further post-operative wound care.

From day 60 after the surgery, fresh blood clots from bladder flushing were noticed every day. Endoscopic examination on day 63 after surgery found the internal lumen of the UB narrow and the presence of red-colored urine with blood clots flowing from the right ureteric canal (Fig. 6). An endoscopic survey into the right ureter indicated that the red color was caused by hemorrhaging from the right kidney. Due to the constriction of the right ureter, the renal pelvis could not be reached. The left ureteric opening was constricted and there was no evidence of urine flowing from this opening.

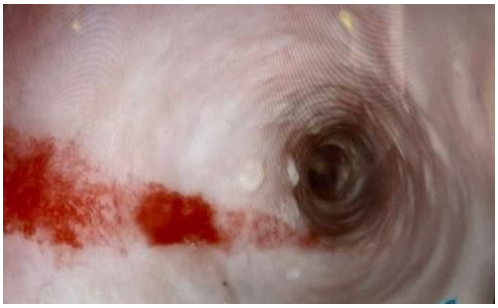

Figure 6 Ureteroscope of the right ureter on day 63 after surgery shows continuous flow of a small blood stream inside the ureter indicating that the continuous hemorrhage is originating from inside the upper urinary tract.

The continuous blood loss from the right kidney corresponded to the hematology results indicating that the PCV had continuously declined. During this time an attempt at blood transfusion was initiated but a compatible donor could not be found. The elephant died on day 80 after the surgery. Cause of death was severe anemia from intra renal hemorrhage, severe weakness and chronic kidney failure.

Urolith analysis: The stone was sent to the Urology Unit of the Veterinary Teaching Hospital, Kasetsart University, Bang Khane Campus for composition analysis. Results from an analysis using the IR Spectrum technique (Uldall, 1981) indicated that the stone was formed by calcium carbonate in both its surface and nuclear regions.

Discussion

The causes of urolith formation in the patient in this case report remains unclear. However, causes may include long-term inadequate water intake, high level of minerals in drinking water, low water content in diet and cystitis. This elephant was translocated from the mainland to an island in the gulf of Thailand where the quality of water and food might not be suitable for long-term consumption. These factors might serve as predisposing factors for urolith formation in the urinary tract. Including this case report, there are only two cases of Asian elephants in Thailand being diagnosed and treated for urolith in the urinary tract and both involved female elephants. The reason why this health condition may occur only in female elephants in Thailand remains unclear and needs further investigation. The urinary tract distal to the UB in male elephants is longer and narrower than that of the female elephant. Theoretically, male elephants should possess a higher risk of urethral calculi. The anatomical characteristic of the urinary tract distal to the neck of UB in female elephant seems to possess low favorable conditions for the development of the nucleus of a stone. Based on induced relaxation of the urethral sphincter by multiple injections of 2% lidocaine hydrochloride previously mentioned in the operation procedure, the intraluminal diameter of the urethral canal in this elephant was approximately 7-10 cm. At this diameter, small stones and an early forming nucleus should pass easily during normal urination and should result in a low incidence of urolith formation. However, this assumption may not accurately reflect health problems related to the urinary tract because in the past several elephants with clinical signs of urinary incontinence were still left undiagnosed and several died without necropsy. Elephant camps on islands prefer to keep only female elephants due partly to their lower aggression and being easier to work with than bull elephants. This may explain why stones in urinary tracts have been diagnosed only in female elephants. The elephant in this case report also showed clinical sign of chronic urinary incontinence, which is a common clinical sign of cystitis. Whether the chronic cystitis in this elephant increased the chance of urolith formation or the presence of urolith in UB leading to cystitis is uncertain. Further investigation and research to elucidate predisposing factors of the formation of stones in the urinary tract of elephants are needed.

Previous case reports of urolith in the urinary tract all involved the urethra region (Morris *et al*., 1987; Ruedi, 1995; Thongtip *et al*., 2013). Surgical removal of multiple small intra-urethral stones via episiotomy and urethrostomy have been reported in this species (Thongtip *et al*., 2013). The female elephant in our case was the first elephant that was diagnosed and received surgical removal of a single large urolith from the UB. For this elephant, the urolith completely obstructed the urethral canal at the neck of the bladder. The urolith was firmly pressed against the internal opening of the UB by high intraluminal pressure from excessive accumulation of urine. However, the obstructed urolith was dislodged from the obstruction site after simple manipulation by a surgeon's hand. This suggests that if complete obstruction of the UB at this region is diagnosed again in female elephants, the affected elephant should be first aided by dislodging the urolith from the obstruction site in order to prevent clinical complications originating from long-term anuria. For example, in this case, necrosis of renal tissues (Fig. 7) led to unstoppable hemorrhaging from the kidney.

Figure 7 Diffuse necrosis of renal tissues of the left kidney (red arrow).

Dilation of the urethral sphincter via blinded infiltration of local anesthesia proved to be feasible and non-invasive. This method is unlikely to impair normal function of the sphincter after complete recovery. However, when fully relaxed, the diameter of the sphincter was still smaller than that of the urolith. Therefore, sphincterotomy was implemented in this case even though the technique is invasive and later impaired the normal function of the sphincter as was seen in this elephant, including continuous urine dripping and significant narrowing of the UB lumen.

There were several factors that had to be overcome in order to remove urolith from the UB of this elephant. First: urolith that were larger than the diameter of the urethral sphincter; this was overcome with a combination of local infiltration with lidocaine and sphincterotomy. Second: urolith kept falling into the

lumen of the UB if released; this was overcome by placing the urolith in the hand-woven basket. Third: negative pressure from the UB that occurred when trying to remove the urolith; this was reduced by inflation bladder with air. The inflation also further stabilized the urolith inside the urethral canal and added positive pressure inside the UB that helped push the urolith caudally. Fourth: a tangle between the surface of the urolith and the connective tissue of the urethral canal; this was gradually removed by insertion of the surgeon's fingers between the urolith and the tangled connective tissues. With all the above mentioned techniques, the large urolith was successfully removed in a live elephant.

A basket to remove a bladder stone was originally used in human patients and has been reported since 1977 (Bapat, 1977; Streit *et al*., 1979; Harrison *et al*., 1983). The hand-woven basket made from 3-mm diameter nylon rope in this case helped stabilize the urolith inside the urethral canal for further manipulation.

The wound from episiotomy was left to heal by secondary intention similar to that of a previous urethrotomy case in a female elephant. During the post-operative period of the previous case, urine was allowed to pass through the incised wound, which later caused accumulation of urine in subcutaneous tissue ventral to the wound and subsequently caused urine scalding and accumulation of urine in subcutaneous tissue of the ventral abdominal wall. In the present case, urine scalding of the subcutaneous tissue around the edge of the wound was minimal and there was no evidence of accumulation of urine in the subcutaneous tissue of the ventral abdominal wall. This might be due partly to the sutures placed between the skin and mucosa of the vestibule that helped prevent leaking of the urine into the subcutaneous tissues. Besides minimal urine scalding, the sutures also facilitated quicker healing. Wounds from uncomplicated episiotomy in elephants usually take a minimum of 6 months to close. Therefore, dehiscence between the vestibule skin and vestibule mucosa usually occurs in 7-14 days after surgery depending on physical activities of the elephant. For this elephant, dehiscence of the wound occurred 14 days after the surgery. Continuous monitoring for possible urine scalding around the wound edge should be continued. Wound cleaning and the UB lavage to remove necrotic tissues were performed daily. Even though the wound edge was exposed to urine during every urination good granulation tissue at the wound edge was seen approximately two weeks after surgery and may have helped to prevent the urine infiltrating into the subcutaneous tissues around the wound.

There are some reports on the high percentage of calcium-based crystals in healthy elephants' urine (Kingsukon *et al*., 2006; Weidner *et al*., 2009; Thongtip *et al*., 2013). Two calcium carbonate stones were found during necropsy in this African elephant that died from chronic renal failure (Morris *et al*., 1987). In this presenting case, during post-operative care, the elephant in this case report developed clinical signs of anemia, hypoalbuminemia and impaired kidney function indicated by an increase in creatinine level.

Based on the size of the urolith from this elephant, the urolith could have obstructed the lower urinary tract earlier. The exact cause that led to the complete obstruction could not be determined. However, possible inciting causes might be chronic inflammation of UB, abnormal bladder tone, abnormal micturition behavior and unusual posture during urination (e.g., urinating when standing in a hilly area). Five days of anuria caused by complete obstruction led to excessive accumulation of urine and increase in pressure inside the urinary tract. This pressure not only injured the tissue of the urinary tract proximal to the obstruction site but might also have damaged the kidney tissue. The retrograde pressure from the UB through the ureter might cause severe ischemic of the renal papillar, renal medulla and renal cortex, which gradually led to necrosis. Severe necrosis of the renal tissue exposed the lumen of the blood vessel in the kidney and caused continuous hemorrhaging of the right kidney. Whether this hemorrhage arose from a vein or artery could not be determined but based on the amount that could be seen during ureteroscope blood might come from small to medium size veins or arteries, such as interlobar, arcuate,or cortical radiated. Significant narrowing of the left ureteric opening and absence of urine flowing from the opening during endoscopic examination on day 63 after the surgery suggested that the left kidney was already completely damaged and no longer able to produce urine.

After intensive post-operative care over a period of a month, clinical signs, blood profiles and serum creatinine level in this elephant had not improved. This elephant died on day 80 after the surgery. A necropsy was performed and the major finding was severe diffuse necrosis of both kidneys. Necrosis and fibrosis of left kidney (Fig.7) and the liquefaction necrosis of right kidney (Fig.8) were found. The anuria caused by the complete obstruction of the bladder for five days in this case interfered with normal kidney function and its blood circulation, and caused acute ischemia, necrosis and kidney failure. Cause of death in this elephant might be a combination of anemia and kidney failure. Evidence of hemorrhage directly from the kidney and the result from the necropsy suggested that acute anuria in elephants requires immediate diagnose and treatment. If complete obstruction is identified in a female elephant, initial first aid such as catheterization with a semi-rigid tube via a small episiotomy window to empty urine may improve the survival rate of elephants suffering from complete obstruction of the urinary tract. Routine ultrasonographic reproductive health examination of a 26-yr-old female African elephant (*Loxodonta africana*) has been reported about the finding of bilateral ureteral wall thickening and dilatation without the signs of urolithiasis. After 30 months, the elephant developed severe signs of lethargy, ventral edema and oral mucosal ulceration. Although blood urea nitrogen remained increased, creatinine, total calcium, and ionized calcium went back to within reference ranges and due to rapid clinical decline and grave prognosis, humane euthanasia was elected. Hydronephrosis of both kidneys and prominent sacculation of the left ureter were noted on ultrasonographic examination without the sign of urolithiasis. Bilateral ureteral

dilatation, dysplasia of the right kidney and chronic nephritis of the left kidney has been found postmortem (Jankowski *et al*., 2012). Then, routine reproductive health examination using trans rectal ultrasonography should be performed in all elephants.

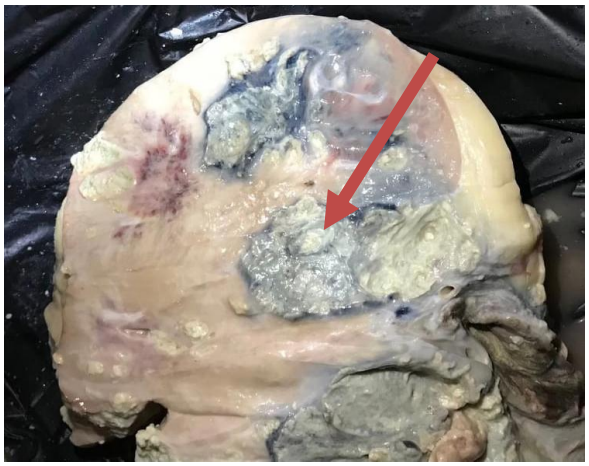

Figure 8 Liquefaction necrosis of the right kidney (red arrow)

This case report suggests that the calcium-based urolith might be common in elephant urine. The high level of calcium salts in the diet and inadequate water intake may have contributed to the formation of calculus in this elephant. Elephants with clinical signs of turbid urine and urinary incontinence should receive further diagnosis for possible health problems related to the urinary tract.

In conclusion, this report documents the authors' experience in the removal of urolith from the urinary bladder in a female elephant. This is the second case in which urolith have ever been removed ante-mortem surgically in a female elephant. The authors are confident that the combination of episiotomy, sphincterotomy and modified basket can be used in future cases to manually retrieve urolith from the urinary bladder in female elephants.

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