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Case report

SUSPECTED UROLITHIASIS IN A FEMALE ZOO YAK (*BOS GRUNNIENS*) BASED ON UROLITH ANALYSIS

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Summary

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Numerous publications devoted to urolithiasis in domestic animals are encountered in the scientific literature. Although less frequently, there are reports on urolithiasis occurrence in wild animals, living either freely in nature or in captivity. Uroliths may form in the urinary tract both of male and female mammals with equal prevalence, yet the clinical disease associated with obstruction or partial obstruction of urinary ducts is much more common in male animals. This is the most probable reason for the lack of scientific reports of spontaneously excreted uroliths in the literature. An accidental finding of four uroliths within a cage facility on the territory of the Stara Zagora Zoo was the incentive for their macroscopic and stereoscopic analysis, as well as powder X-ray diffraction (XRD) analysis of the phase composition of the samples. The stones were identified as struvite, composed of magnesium ammonium phosphate ($MgNH_4PO_4.6H_2O$). Our hypothesis was that they were excreted by a female yak (*Bos grunniens*) reared in the facility because of their size and some specific biological features of yaks.

Key words: *Bos grunniens*, captive, struvite stones, urolithiasis, yak, zoo animals

Some mineral solutions precipitate and form crystals in urine. These crystals may aggregate assuming macroscopic size, forming uroliths (calculi or stones). In general, uroliths contain organic matrix whose proportion varies from 2% to 10% of their chemical composition. The residual 90–98% of urolith's composition comprise different minerals depending on its type. Urolithiasis can be defined as the formation of sediment anywhere within the urinary tract (Ulrich *et al.*, 1996; Tion *et al.*, 2015). Uroliths may develop in the kidney, the ureter, the urinary bladder or the urethra and are respectively termed nephroliths, ureteroliths, urocystoliths and urethroliths (Brown, 2022).

The etiology of urolithiasis is multifactorial: acid-base balance of the diet, potassium and water intake and early castration (Mashwani *et al.*, 2012). Uroliths can form in the urinary tract of both male and female mammals. Females are less likely to be affected due to a urethra that is shorter in length and larger in diameter compared to the male urethra. Thus, any calculi that form may be easily passed. Males are more prone to the disease due to the presence of a sigmoid flexure of the urethra. Bulls castrated at an early age lack the testosterone necessary to further develop the size of the penis and the diameter of its urethra and are more prone to blockages (Onmaz *et al.*, 2012; Armstrong, 2023).

The scientific reports devoted to urolithiasis in domestic animals – cattle, buffaloes, horses, sheep, goats, pigs, dogs and cats; its treatment, chemical composition of uroliths and causes of onset are numerous (Rao & Unmack, 1973; Parrah *et al.*, 2010; Onmaz *et al.*, 2012; Makhdoomi & Gazi, 2013; Tion *et al.*, 2015; Brown, 2022; Jones, 2022a,b; Valkova *et al.*, 2023). Although less frequently, cases of urolithiasis in wild and captive animals – red deer (*Cervus elaphus*) (Reynolds, 1982), brown bear (*Ursus arctos*) (Hubenov *et al.*, 1999), reticulated giraffe (*Girafa camelopardalis reticulata*) (Wolfe *et al.*, 2000), maned wolf (*Chrysion brachiurus*), (de Oliveira Fernandes & Marcolino, 2007), Eurasian otters (*Lutra lutra*), Asian small-clawed otters (*Aonix cinerea*) (Bochmann *et al.*, 2017), Siberian chipmunk (*Eutamias sibiricus*) (Kohutova & Jekl, 2021) are also reported.

Obstructive and incidental urolithiasis cases were evaluated in 5 Asian colobine monkey species (21 individuals) that included 12 silvery langurs (*Trachypithecus cristatus*), 6 spectacled langurs (*Trachypithecus obscurus*), one Javan langur (*Trachypithecus auratus auratus*), one François' langur (*Trachypithecus francoisi*), and one red-shanked douc langur (*Pygathrix nemaeus*) from eight zoologic institutions (Baker *et al.,* 2022). In guinea pigs (*Cavia porcellus*) urolithiasis has been reported by Azvedo *et al.* (2023). Liptovszky *et al.* (2014) were the first to report recurrent appearance of urolithiasis in a captive group of Tammar wallabies (*Macropus eugenii*). Uroliths are a rare finding in amphibians, although Archibald *et al.* (2015) reported such in captive waxy monkey frogs (*Phyllomedusa sauvagii*).

The literature overview confirmed the studies of Osborne *et al.* (2009) that urolithiasis is encountered in herbivores, carnivores, exotic and wild animals. The aim of the present study was to investigate the structure, chemical composition and the origin of the findings.

Case description

This report deals with an accidental finding of four uroliths within a cage facility on the territory of the Stara Zagora Zoo. They differed from all other stones on the ground by their shape, color, size and hardness. This allowed assuming that they were of biological origin and raised our interest to investigate the stones in order to discover their origin.

The cage facility covered an area of 5.5 thousand m^2 on a natural terrain and housed a mixed population of non-antagonistic mammals and birds – mouflons (*Ovis musimon*), goats (*Capra hircus),*

Fig. 1. View from the mixed animal population, Stara Zagora Zoo.

Karakachan sheep, a female yak (*Bos grunniens*), wild American turkeys (*Meleagris gallopavo*) and ostriches (*Struthio camelus*) (Fig. 1).

The shape of findings varied from pyramidal with rounded borders and tips to ovoid. When a stone was broken, its layered crystal structure was macroscopically visible which suggested that the findings were uroliths. The uroliths were photographed with iPhone 10 Designed by Apple in California, Assembled in China. Metric measurements of calculi were performed by means of a digital caliper (Neiko 01407A Stanless Steel 6-Inch Digital Caliper with Extra-Large LCD Screen and Instant SAE-Metric Conversion), with precision of 0.01 mm (Michaylov & Dimitrov, 2010, Mihaylov *et al.*, 2013). Weight measurements were performed with „Sartorius "– PT 120 electronic balance with precision of 0.01 g.

The observations and documentation of the internal view of broken uroliths were conducted with a stereomicroscope МБС–10 (USSR) and digital stereomicroscope (Leica S9i 10×23, Microsystems, Switzerland), camera TM69 (Leica S9i Stereozoom) and analysis software (LAS X 5.1.0, Leica Microsystems, Switzerland).

The phase composition of the samples was investigated by powder X-ray diffraction (XRD) analysis. The powder XRD patterns were recorded on a diffractometer D8 Advance, Bruker. Filtered Co-K_ radiation was used in the range 2Q 4–80_, step 0.02^{o} 2Q, and exposure time per step 1.5 s. The specialised software Diffrac.EVA version 5.2.0.5 was used for qualitative and semi-quantitative phase composition determination. The stone that was found already broken was not investigated.

The shape of sample 1 (Fig. 2, right) was that of an almost regular tetrahedron, with wall sizes of 26.7 mm, 27.1 mm and 27 mm, and height of 27 mm. Its colour was off-white and hard as a chalk. The shape of sample 2 (Fig. 2, left) was that of quadrilateral pyramid with wall sizes of

Fig. 2. Sample 1 (right), sample 2 (left), sample 3 (in the middle); the broken urolith is in front of them.

39.8 mm, 35.3 mm, 32.4 mm and 34.1 mm. Its height was 34.2 mm. The stone color was light gray and its hardness – identical to that of sample 1. Sample 3 (Fig. 2, middle) was of the largest size and had a shape similar to that of sample 2, but due to the more rounded borders, it was assumed to be ovoid. The widest part of the stone was 43.8 mm, and its height was 62.1 mm. The stone color was gray; its hardness was similar to the other 2 uroliths. Struvite calculi in the urinary bladder of similar shape were demonstrated by Breshears & Confer (2017) and Brown (2022). Parrah *et al.* (2010) reported three cases in male calves with calculi 37 to 59 mm of size and weight between 35 and 65 g isolated from the urinary bladder lumen. Weight measurements of stones demonstrated respective weights of 16.84 g for sample 1 (Fig. 3), 49.70 g for sample 2 and 110.85 g for sample 3 (Fig. 4).

After the samples were measured and weighed, they were partially broken for stereomicroscopic examination (Fig. 5, 6 and 7). Stereomicroscopy of samples showed the presence of an obvious central homogeneous nucleus of spherical shape and green-yellowish colour inside sample 1 (Fig. 5) and sample 2 (Fig. 6). The periphery had a marked crystal structure. In the middle of the periphery of sample 2, there was a concentric brown band resembling a tree ring (Fig. 6). Unlike the other 2 samples, in the centre of sample 3 (Fig. 7) there were large crystals instead of a round nucleus. In the periphery of sample 3, concentric circles of various tints and widths were clearly seen (Fig. 7). Around 4 out of 5 circles were brown, resembling the rings of a tree similarly to sample 2 (Fig. 6).

Presuming that uroliths are body products, it may be hypothesised that the concentric bands of different colour observed in samples 2 and 3 were function of the physiological status of the animal and/or seasonal changes. Stereomicroscopy of samples demonstrated clearly that their general structure was compatible to that of uroliths. They were made of a matrix of biological origin, described by some authors as „*nidus*" with subsequent deposition of inorganic minerals, including magnesium, calcium and phosphorus on it (Jones & Miesner, 2009; Lulich *et al.*, 2010). However, the opinion of Manzoor *et al.* (2019) that the diverse microstructure of similar and other types of stones proves that their formation is complex and may be managed by both physiological and microenvironmental factors should be also taken into consideration.

Fig. 3. Weighing sample 1.

Fig. 4. Weighing sample 3.

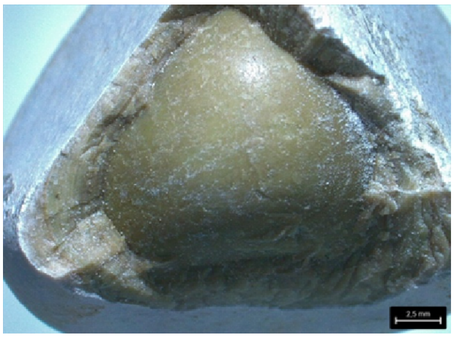

Fig. 5. Stereoscopy, sample 1.

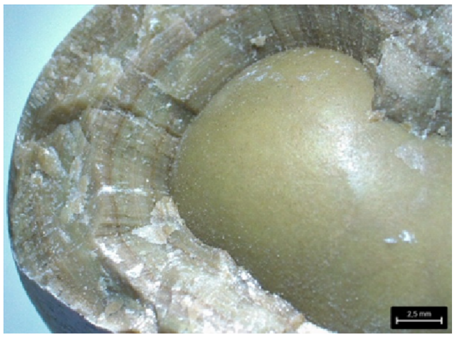

Fig. 6. Stereoscopy, sample 2.

Fig. 7. Stereoscopy, sample 3.

The results from the powder X-ray diffraction analysis (Fig. 8) showed that the chemical composition of the periphery and the “nucleuswas the same for the three samples (Table 1). The analysis determined that the main constituent of the three samples was magnesium ammonium phosphate hexahydrate (struvite or $MgNH_4PO_4(H_2O)6$), which is commonly encountered in ruminants (Rao & Unmack, 1973; Parrah *et al.*, 2010; Onmaz *et al.*, 2012; Makhdoomi & Gazi 2013). The proportion of MgO in the periphery and the “nucleus” of the three samples ranged from 16.42% to 18.56% (Table 1), whereas that of P_2O_5 – from 22.86% to 26.27%. According to Matsui (2007), excess of Mg is one of factors causing urolithiasis in cats and cattle and enterolithiasis in horses, however, the magnesium level in practice diets alone is not able to induce disease. Urolithiasis in cattle and enterolithiasis in horses develop in combination with high phosphorus intake. We assumed that the natural sward within the facility where the animals graze was one of possible factors predisposing to high magnesium dietary intake.

In the scientific literature, numerous cases of obstructive urolithiasis have been reported (Rao & Unmack, 1973; Reynolds, 1982; Hubenov *et al.*, 1999; Wolfe. *et al.*, 2000; de Oliveira Fernandes & Marcolino, 2007; Parrah *et al.*, 2010; Onmaz *et al.*, 2012; Makhdoomi & Gazi, 2013; Brown, 2022; Jones, 2022a,b; Valkova *et al.*, 2023). Most of them are in male animals, and although females are also affected, yet the cases of the disease in them are few (Smith *et al.*, 1983; Onmaz *et al.*, 2012).

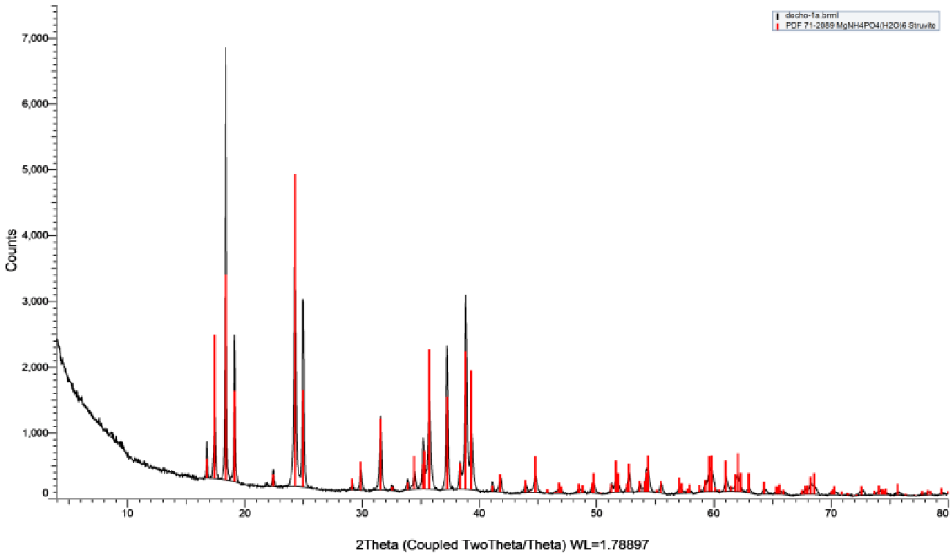

Fig. 8. Powder X-ray diffraction to determine the type of urolith.

Table 1. Chemical composition of the uroliths (%)

Composition (%)	Sample 1		Sample 2		Sample 3	
	periphery	centre	periphery	centre	periphery	centre
Na_2O	0.05	0.05	0.04	0.05	0.04	0.16
MgO	18.56	18.33	18.53	18.50	18.55	16.42
Al_2O_3	0.05	0.06	0.03	0.02	0.03	0.58
SiO_2	0.51	0.59	0.35	0.36	0.33	6.26
P_2O_5	26.27	25.99	26.02	26.04	25.99	25.02
K_2O	1.25	1.22	1.10	1.18	1.05	1.17
CaO	0.10	0.08	0.06	0.06	0.06	1.31
TiO_2	0.01	0.01	0.01	0.00	0.00	0.07
MnO	0.02	0.02	0.02	0.02	0.02	0.02
Fe_2O_3	0.09	0.08	0.08	0.06	0.09	0.37
LOI*	53.09	53.57	53.74	53.70	53.82	50.76
Total	99.98	99.98	99.98	99.98	99.98	99.96

*LOI - lost on ignition.

To the best of our knowledge, no reports about spontaneously excreted uroliths were found in available literature, most probably because the condition was not accompanied with clinical signs. In support of this, Onmaz *et al.* (2012) concluded that the post mortem physical examination of 1,840 cows without clinical abnormalities revealed uroliths in 20 animals (1.1%). We found it important to discover the animal that has excreted spontaneously the uroliths found on the ground of the Stara Zagora Zoo. It was assumed that because of samples' size, especially that of sample 2 and the ovoid sample 3, the stones would hardly pass through the long and narrower urethra with sigmoid flexure and the urethral process of male animals, housed in the facility – mouflons, bucks and Karakachan rams, in line with the opinion of other researchers (Smith *et al.*, 1983; Parrah *et al.*, 2010; Makhdoomi & Gazi, 2013; Jones, 2022a, Armstrong, 2023).

Our hypothesis was that most probably, the detected struvites have been excreted by a female large mammal, housed in the fenced area. This was substantiated by the anatomical features of female excretory system in ruminants – short urethra without curvatures, which is also wider and allows greater broadening than the male urethra, whose enlargement is limited by the penile body. The large size of sample 2: width – 39.8 mm; length – 34.1 mm and sample 3: width – 43.8 mm; length – 62.1 mm allowed presuming that female animals the size of a mouflon, goat or Karakachan sheep could not excrete these stones, so we supposed that they were excreted by the female yak (*Bos grunniens*) from the zoo collection because of its larger body weight compared to the other animals housed within the cage facility.

Houston *et al.* (2004) reported a struvite stone occupying bladder weighing 0.6 kg, length 125 mm and width 75 mm in an adult sterilized female Border Collie cross, removed by operative intervention.

The domesticated yak (*Bos grunniens*) is not a species, specific for Bulgarian fauna. Some representatives are found in the collections of several national zoos – in Stara Zagora, Lovech and Sofia. After the attempt for introduction during the 1980s, a total of 164 yaks imported from Mongolia have been introduced and resettled at different locations. At present, the only place at the Balkan Peninsula where 25–30 yaks live in the wild at an altitude of <1800 m, is the Vitinya State Hunting Preserve, Bulgaria (Vasilkovski, 2023). The yak (*Bos grunniens*), an indigenous herbivore raised at altitudes between 3 000 and 5 000 m above sea level across the Asian highlands, is the main livestock grazing on the highlands, particularly the vast rangelands of the QTP (the Qinghai-Tibetian Plateau) and regions around the Himalayan Mountain range. The yaks have developed adaptation strategies including genetic, morphological, physiological, metabolic, etc. features distinguishing them from cattle by reason of the harsh life at a high altitude (Leslie & Schaller, 2009, Ayalew *et al.*, 2021, Jing *et al.*, 2022). These features include the long coat, 14 thoracic vertebrae, a shorter tongue, mobile lips to graze short grasses like sheep, etc. In relation with the adaptation strategies of yaks, some authors (Leslie & Schaller, 2009, Ayalew *et al.*, 2021, Jing *et al.*, 2022) reported that in the natural environment they assimilated and retained nitrogen to a greater extent than native cattle having a lower urinary nitrogen excretion rate (a possible mechanism of adaptation to insufficient food supply) and a more efficient utilisation of nitrogen, which is at least partly due to the greater rumen production of microbial protein.

This led us to the conclusion that the unnatural environment at a low altitude of 196 m, at which the Stara Zagora Zoo is located, with four seasons instead of two (one of which the long and nutrient-deficient winter), available grass for grazing in the facility with regular provision of protein-rich feed – alfalfa hay, wheat, barley, fruits and vegetables, and the yaks' "nitrogen-sparing" strategy (Leslie & Schaller, 2009; Ayalew *et al.*, 2021; Jing *et al.,* 2022) are predisposing factors for the formation of struvites by the female yak in the Stara Zagora Zoo, most likely excreted from the animal and found in the facility. When a mammal consumes a relative excess of digestible protein, the absorbed amino acids are degraded and the amino group are removed as urea through the kidneys. Urea is easily converted to ammonia, one of the building blocks of struvite. This confirms the research results of Jones & Miesner (2009) that struvites can often be observed in animals fed a high-cereal ration, similar to the diet of the female yak in Stara Zagora Zoo.

CONCLUSION

It was assumed that the struvite uroliths in the female yak (*Bos grunniens*) kept in the Stara Zagora Zoo were formed for several reasons. First of all, the animal was kept at a much lower altitude compared to its natural habitat, where food resources are scarce. Second, the almost year-round presence of green grass for grazing in the facility suggests an increased dietary intake of magnesium. Third, the "nitrogen-saving" strategy of yaks in this case turned out to be unnecessary, since food for the animals at the zoo was provided without limitation year-round which perhaps was another reason for the formation of struvites. Last, another prerequisite for the formation of struvites is the high content of cereal crops in the diet of the female yak in the zoo.

On the basis of these conclusions, we recommend adjustment of the nutrition regimes of yaks (*Bos grunniens*) reared in zoos and elsewhere in unnatural environments, considering their biological, morphological and physiological characteristics and one of their high-altitude survival strategies e.g. the "nitrogen-sparing" strategy.

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