



## SHORT COMMUNICATION

### Studies on Electron Microscopy of Uroliths in Dogs

P Sravanthi<sup>1</sup>, EL Chandra Sekhar<sup>1</sup>, KBP Raghavender<sup>2</sup> and D Pramod Kumar<sup>3</sup>

<sup>1</sup>Department of Veterinary Surgery & Radiology; College of Veterinary Science, Korutla, Karimnagar 505 326 , Telangana State, India; <sup>2</sup>Department of Veterinary surgery & Radiology; <sup>3</sup>Department of Veterinary Anatomy & Histology, Collge of Veterinary Science, Rajendra nagar , Hyderabad- 520 030, Telangana , India

#### ARTICLE INFO

Received: June 07, 2014  
Revised: June 14, 2014  
Accepted: August 03, 2014

#### Key words:

Dog  
Ultrastructure  
Uroliths

#### \*Corresponding Author

P Sravanthi  
sravanthivet@gmail.com

#### ABSTRACT

A study on Electron Microscopy of the uroliths was conducted in 9 clinical cases in dogs. Analysis of the calculi revealed calcium oxalate calculi in 55.56 per cent of the dogs, magnesium ammonium phosphate calculi in 33.33 per cent and calcium oxalate plus urate calculi in 11.11 per cent of the dogs. Scanning electron microscopy revealed bar shaped crystals in calcium oxalate calculi, rock like crystals in magnesium ammonium phosphate calculi and spindle shaped crystals in calcium oxalate plus urate calculi.

**Cite This Article as:** Sravanthi P, ELC Sekhar, KBP Raghavender and DP Kumar, 2014. Studies on electron microscopy of uroliths in dogs. *Inter J Vet Sci*, 3(4): 177-180. www.ijvets.com

## INTRODUCTION

Urolithiasis is a disease of multi factorial origin. Factors like diet, age, sex, breed, genetic makeup, season, mineral and infection play role in the genesis of urolithiasis (Osborne *et al.*, 1986; Hoppe, 1998; Fazili and Ansari, 2007). The major mineral component of calculi in urinary bladder was reported to be struvite and that in the urethra was calcium oxalate (Kim Chaewook *et al.*, 2004). Super saturation of urine with calculogenic substances has been reported to be an important driving force behind urolith formation (Bartges and Lane, 2003). Sustained alteration in the urine composition promoted super saturation of one or more substances eliminated in urine and resulted in their precipitation and subsequent growth. The microscopic evaluation of the shapes of mineral crystals represents only a tentative index of their composition because variable conditions associated with their formation, growth and dissolution may alter their appearance. The present study was conducted to study the electron microscopy of the calcium oxalate, magnesium ammonium phosphate calculi and combined calcium oxated and urate calculi collected from the clinical cases.

## MATERIALS AND METHODS

The calculi were collected after surgery from nine clinical cases of urolithiasis and quantitative chemical analysis was done by using scanning electron microscopy

(Domingo Neumann *et al.*, 1996). For scanning electron microscopy samples were fixed in 2.5% Gluteraldehyde in 0.1M phosphate buffer (pH 7.2) for 24 hrs at 4°C and post fixed in 2% aqueous osmium tetroxide for 4 hrs, in the same buffer. After the post fixation samples were dehydrated in series of graded alcohols and dried to critical point drying with electron microscopy science critical point drying unit. The dried samples were mounted over the stubs with double-sided carbon tape. Applied a thin layer of gold coat over the samples by using an automated sputter coater (JEOL JFC-1600) for 3 min. Then scanned the samples under scanning electron microscope (Model: JOEL-JSM 5600) at various magnifications at RUSKA Labs, College of Veterinary Science, Rajendranagar, Hyderabad. Prior to subjecting the calculi to scanning electron microscopy, they were examined under high power microscope.

## RESULTS AND DISCUSSION

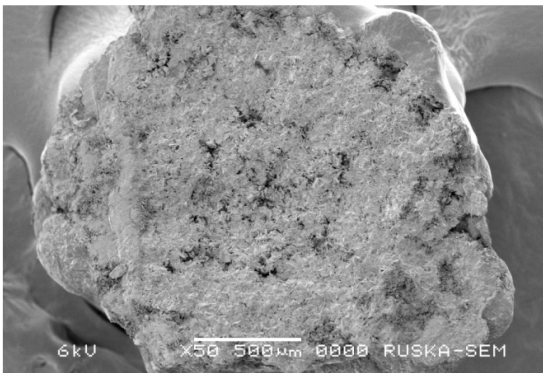
Analysis of calculi revealed that the majority of calculi were made up of calcium oxalate, accounting to 5 (55.56%) cases. Among the rest, 3 (33.33%) cases comprised of magnesium ammonium phosphate calculi and in 1 (11.11%) case, the calculi comprised of calcium oxalate and ammonium urate/uric acid. Calcium oxalate uroliths, on physical examination appeared hard and brittle with sharp edges protruding from the surface and magnesium ammonium phosphate calculi were yellow to



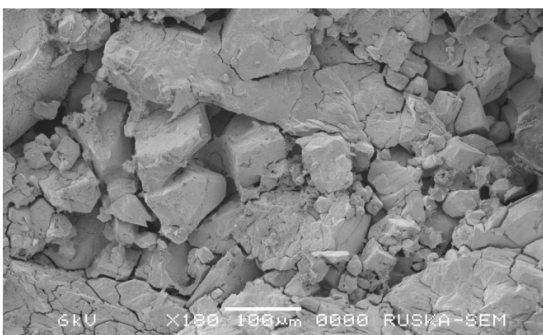
**Fig. 1:** Photomicrograph of the calcium oxalate plus ammonium urate calculi showing nucleus in the center and concentric zones around it.



**Fig. 2:** Scanning electron micrograph showing bar shaped calcium oxalate crystals (note the porosity in the calculi)



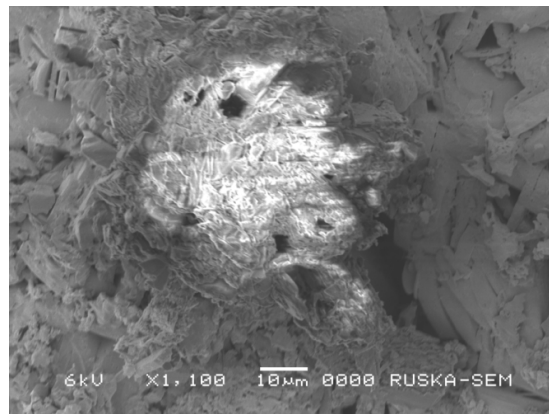
**Fig. 3:** Scanning electron micrograph showing calcium oxalate crystals sizes which are smaller towards the periphery (note the smooth surface).



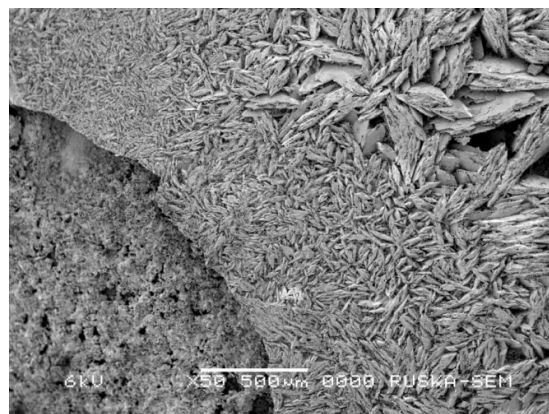
**Fig. 4:** Scanning electron micrograph showing irregularly arranged rock like magnesium ammonium phosphate crystals.



**Fig. 5:** Scanning electron micrograph showing large sized magnesium ammonium phosphate crystals even towards the periphery.



**Fig. 6:** Scanning electron micrograph of the magnesium ammonium phosphate calculi showing the organic matrix and crystallization around this (arrow)

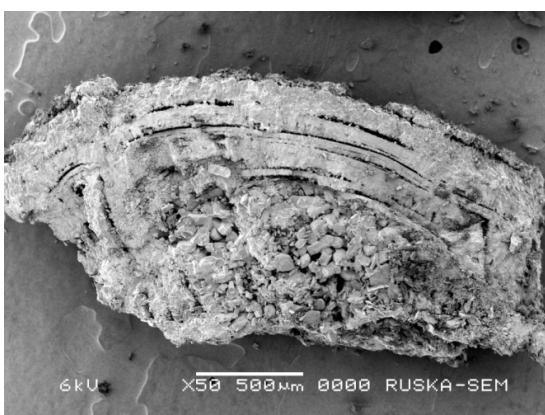


**Fig. 7:** Scanning electron micrograph showing spindle shaped urate crystals. Note that the crystals are larger towards the periphery. The demarcation between calcium oxalate and urate crystals is very clear (arrow)

white in colour and fairly hard and easily crushed to chalky powder whereas calcium oxalate and ammonium urate calculi were found to be yellow in colour, brittle and smooth. Prior to subjecting the calculi to scanning electron microscopy, they were examined under high power microscope. Microscopic examination of calcium



**Fig. 8:** Scanning electron micrograph showing area of organic matrix in the calculi (arrow)



**Fig. 9:** Scanning electron micrograph of calcium oxalate plus urate showing the areas of crystallization arranged in eggshell like laminations in concentric zones

oxalate plus ammonium urate calculi revealed a central small white coloured area that formed the nucleus or the matrix for the formation of urinary calculi. Around this small nuclear area, calculi were observed to have formed in concentrically arranged zones (Figure 1).

### Scanning electron microscopy

#### Calcium oxalate calculi

Scanning electron microscopy of the oxalate calculi revealed that the crystals that went into formation of the calculi were elongated and bar shaped and were arranged in such a way that there was porosity in the calculi (Figure 2), leading to capillary formation. It was observed that the crystals were arranged in concentric layers comprising of several zones. There was distinct demarcation between the different zones. As the calculi were scanned towards the periphery by scanning electron microscopy, it was observed that towards the surface, the crystal size became smaller until the surface was very smooth with very small crystal size (Figure 3). Domingo-Neumann *et al.* (1996) described four texture types for oxalate and phosphate calculi each. They also found porosity between the oxalate crystals. They further reported that the minute pores increased the surface area of calculi exposed to urine, and this increase in liquid-solid interface promoted interaction of crystals with the surrounding urine. In the present study no

organic matrix or cell debris was noticed in the oxalate calculi. However, Ebisuno *et al.* (1997) reported sporadic finding of some complexes that consisted of aggregated calcium oxalate crystals and cell debris on scanning electron microscopy.

#### Magnesium ammonium phosphate calculi

Scanning electron microscopy of these calculi revealed that the crystals that formed the calculi were irregular in shape, were of varying sizes and were irregularly arranged (Figure 4). The rock like crystals were arranged close to each other. Towards the periphery of the calculi, the crystals were large in size, but the surface of the calculi was found to be smooth (Figure 5). In these calculi, the crystals were seen to be arranged irregularly and not in any specific concentric zones. In addition, scanning microscopic examination of the calculi also revealed several areas that indicated either cell debris or bacterial cells, called as organic matrix, and it was observed that there was crystallization around these zones of debris (Figure 6). It was therefore noticed that within an individual calculus, there were multiple nidi or nuclei leading to formation of the calculus. The rock like crystals were arranged close to each other in specific concentric zones. Shaw and Sherri (1997) also made similar observations.

#### Calcium oxalate plus ammonium urate calculi

Scanning electron microscopy of these calculi revealed that the urate crystals that formed the calculi were spindle shaped with sharp edges on either sides. The spindle shaped crystals were of varying sizes with the smaller crystals towards the center and larger crystals towards the periphery (Figure 7). It was found that the ammonium urate crystal zone was very clearly and distinctly demarcated from the oxalate zone. These calculi also showed some areas of organic matrix (Figure 8). Study at lower magnification under the scanning electron microscope revealed many distinct egg shell like laminations or areas of crystallization arranged in concentric zones (Figure 9) indicating that the crystal deposition probably occurred periodically and there was possibly a period of quiescence between periods of crystal deposition in the calculi.

Urolith formation was not a specific disease, but the sequelae to a group of disorders which promote super saturation of one or more substances eliminated in urine and resulted in their precipitation and subsequent growth. The degree of super saturation might be influenced by the magnitude of renal excretion of the crystalloid, urine pH and crystallization inhibitors in urine (Lulich and Osborne, 1995). Shaw and Sherri (1997) stated that uroliths were thought to form from precipitation crystallization supersaturation of mineral components in urine), matrix nucleation (precipitation of minerals around a preformed organic nidus) or crystallization inhibition (the absence of inhibitors in the urine leading to mineral precipitation). The microscopic evaluation of the shapes of mineral crystals represents only a tentative index of their composition because variable conditions associated with their formation, growth and dissolution may alter their appearance. In conclusion scanning electron microscopy was found to be helpful in identification of the crystal type and its deposition around the nuclei which

will give identification about the composition of minerals so that proper dietary as well as managerial practices can be adopted to manage the urolithiasis in canines as a preventive measure.

#### REFERENCES

- Bartges JW and Lane IF, 2003. Medical treatment of urolithiasis. *In: Textbook of Small Animal Surgery Vol 2, III ed.* Elsevier Science, USS pp: 1661-1671.
- Domingo-Neumann RA, AL Ruby, GV Ling, PS Schiffman and DL Johnson, 1996 Ultrastructure of selected struvite containing urinary calculi from dogs. *Amer J Vet Res*, 62; 237-247.
- Ebisuno, Kohjimotoy, M Tamura, Inagaki and T Ohkawa, 1997. Histological observations of the adhesion and endocytosis of calcium oxalate crystals in MDCK cells and in human kidney. *Urol Inter*, 58: 227-231.
- Fazili MR and MM Ansari, 2007. Prevalence of bovine obstructive urolithiasis in Kashmir valley. *Ind Vet J*, 84: 540-541.
- Hoppe A, 1998. Urolithiasis in dogs *In: Waltham Focus-Focus on the urinary tract.* Waltham Inc London, UK, pp: 50-57.
- Kim-Chaewook, Choi-Ulsoo, Chegal-Jun, B Bokyoung, L Doohyoung, Koyoung Hwan and LW Chang, 2004. Canine urolithiasis, interrelation between breed, age, sex, anatomic location, urine pH, crystal and mineral composition of uroliths (270 cases). *J Vet Clinics*, 21: 264-269.
- Lulich JP and CA Osborne, 1995. Canine calcium oxate uroliths. *In: Bonagura JD, GV Ling, CE Franti, DL Johnson and AL Ruby, 1998. Urolithiasis in dogs-III. Prevalence of urinary tract infection and interrelationship of infection age, sex and mineral composition.* *Amer J Vet Res*, 59: 643-649.
- Osborne CA, CW Clinton and LK Bamman, 1986. Prevalence of canine urolithiasis. *Vet Clinics of North Amer Small Anim Prac*, 16: 27-44.
- Shaw DH and LI Sherri, 1997. Urinary tract diseases and Fluid and electrolyte disorders. *In: Small Animal Internal Medicine I ed.* Williams and Wilkins, USA, p: 113.