Chemical Composition and Scanning Electron Microscopic (SEM) Aspects of Uroliths in Geriatric Dogs

Jupaka Shashank*, Ph.D. Department of Veterinary Medicine, C.V.Sc., Rajendranagar, Hyderabad, PVNR TVU, Telangana, India.
K. Satish Kumar, Professor and University Head, Department of Veterinary Medicine, C.V.Sc., Rajendranagar, Hyderabad, PVNR TVU, Telangana, India.
V.V.V. Amruth Kumar, Associate Professor and Head, Department of Veterinary Medicine, C.V.Sc., Mamnoor, PVNR TVU, Telangana, India.
B. Anil kumar, Assistant Professor, Department of Veterinary Pharmacology and Toxicology, C.V.Sc., Rajendranagar, Hyderabad, PVNR TVU, Telangana, India.
M. Lakshman, Professor and Head, Department of Veterinary Pathology, C.V.Sc., Rajendranagar, Hyderabad, PVNR TVU, Telangana, India.

Corresponding Author: K. Satish Kumar

Abstract

Out of the total 246 geriatric dogs with lower urinary tract disease (LUTD) in the present study, 71 dogs were diagnosed for the presence of uroliths of various size and shape and their location, using x-ray and ultrasonography. Out of these, the most prevalent anatomic locations of caliculi were in the urinary bladder, urethra in males, and majorly urinary bladder in females. The caliculi that were detected on x-ray, and ultrasonography were retrieved surgically and processed for chemical analysis, and subjected to scanning electron microscopy. Various caliculi that were investigated with scanning electron microscopy (SEM) revealed, perpendicularly cracked fragments showed concentric laminations composed of compact and loosely packed strata alternately as magnesium ammonium phosphate uroliths, surface of eggshell-like fragments exhibited the scattered hexa-hedral coffin lid-shaped crystals upon the numerous spherular crystals at center, towards periphery and periphery areas, irregularly arranged rock like structures as large and small sized, and large sized regular magnesium ammonium phosphate uroliths, surfaces of few calcium phosphates stones were cracked like egg shells, calcium oxalate monohydrate uroliths were noticed as ‘picket fence appearance’, and bipyramidal shape in calcium oxalate dihydrate crystals on scanning electron microscopy (SEM).

Keywords: Scanning Electron Microscopy, Magnesium ammonium phosphate, Calcium oxalate, Calcium phosphate, Geriatric dogs.

1. Introduction

Urolithiasis is a general term referring to the causes and effects of stones anywhere in the urinary tract that should not be viewed conceptually as a single disease with a single cause, but rather as a sequela of multiple interacting underlying abnormalities. Thus, the syndrome of urolithiasis may be defined as the occurrence of familial, congenital, or acquired pathophysiologic factors that, in combination, progressively increase the risk of precipitation of excretory metabolites in urine to form stones or uroliths (Fazili and Ansari, 2007; Kim *et al.*, 2004). Supersaturation 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 supersaturation of one or more substances eliminated in urine and resulted in their precipitation and subsequent growth. The major mineral component of calculi in the urinary bladder was reported to be struvite and that in the urethra was calcium oxalate (Kim *et al.*, 2004). The microscopic evaluation of the shapes of mineral crystals sometimes represents a tentative index of their composition because variable conditions associated with their formation, growth, and dissolution may alter their appearance. The present study was undertaken to study the scanning electron microscopy of the magnesium ammonium phosphate calculi, calcium oxalate, and calcium phosphate calculi collected from the clinical cases.

2. Materials and Methods

Various types of crystals found in the urine sediment and the various uroliths (stones) removed from the bladder and urethra of geriatric dogs that were presented to the nephrology ward of VCC, CVSc., Rajendranagar, Hyderabad were subjected to chemical analysis. Later these calculi were subjected for ultramicroscopic aspects using scanning electron microscopy (SEM). The material was isolated under stereozome, mounted on double-sided sticky carbon tape, and exposed/fixed with 1 % osmium tetroxide as a fume fixation. The processed samples were mounted over the stubs with double-sided carbon conductivity tape, and a thin layer of gold coat over the samples was done by using an automated sputter coater (Model-JEOL JFC-JSM 5600) at required magnification as per the standard protocol (John and Lonnie, 1998; Lakshman, 2017 and Lakshman, 2019), at RUSKA laboratory, college of Veterinary Science, Rajendranagar, Hyderabad.

3. Results

Chemical analysis of the different stones (uroliths) retrieved from the urinary bladder and urethra revealed magnesium ammonium phosphate (struvite), calcium oxalate, carbonate apatite (calcium phosphate), and sodium urate. The ultrastructural characteristic aspects of these magnesium ammonium phosphate, calcium oxalate and calcium phosphate was done with scanning electron microscopy.

Magnesium ammonium phosphate uroliths (Struvite)

When topographic features of various caliculi were investigated with scanning electron microscopy (SEM), perpendicularly cracked fragments with concentric laminations composed of compact and loosely packed strata alternately (Fig. 1) suggesting struvite which was confirmed on chemical analysis as consisting to be magnesium ammonium phosphate (MAP). The surface of eggshell-like fragments exhibited the scattered hexahedral coffin lid-shaped crystals upon the numerous spherular crystals at the center, towards the periphery and periphery areas (Fig. 2-4). Irregularly arranged rock-like structures as large and small-sized, and large-sized regular magnesium ammonium phosphate uroliths (Fig. 5 - 8).

Calcium phosphate uroliths (Carbonate apatite)

Calcium phosphate crystals are commonly found as a minor component within struvite and calcium oxalate uroliths and are more likely at high urine pH (>7.5). Calcium phosphate uroliths occur in the same breeds as calcium oxalate uroliths, and the risk factors are similar (including primary hyperparathyroidism, other hypercalcemia disorders, renal tubular acidosis, idiopathic hypercalciuria, excessive dietary calcium and phosphorus ingestion). In the present study, the surfaces of a few stones were cracked like egg shells (Fig. 9 and 10) and revealed numerous calcium phosphates on chemical analysis.

Calcium oxalate uroliths

In the present study calcium oxalate monohydrate uroliths which were confirmed on chemical analysis were noticed as 'picket fence appearance' (Fig. 11) and calcium oxalate monohydrate small crystals (Fig.12). Whereas, calcium oxalate dihydrate crystals on scanning electron microscopy (SEM) were seen as bipyramidal shape (Fig. 13) with porosity in the caliculi (Fig. 14).

4. Discussion

When topographic features of various caliculi were investigated with scanning electron microscopy (SEM), perpendicularly cracked fragments showed concentric laminations composed of compact and loosely packed strata alternately suggesting struvite which was confirmed on chemical analysis as consisted to be magnesium ammonium phosphate (MAP). The surface of eggshell-like fragments exhibited the scattered hexa-hedral coffin lid shaped crystals upon the numerous spherular crystals at center, towards periphery and periphery areas**.** Irregularly arranged rock-like structures of magnesium ammonium phosphate urolith findings are in agreement with Shaw and Sherri 1997, Suzuki *et al.* 1997, Sravanthi *et al.* 2014b, and Kumar and Srikanth 2021. Calcium phosphate crystals are commonly found as a minor component within struvite and Calcium oxalate uroliths are more likely at high urine pH (>7.5). Calcium phosphate uroliths occur in the same breeds as calcium oxalate uroliths, and the risk factors are similar (including primary hyperparathyroidism, other hypercalcemia disorders, renal tubular acidosis, idiopathic hypercalciuria, excessive dietary calcium and phosphorus ingestion) (Lulich *et al.,* 2000 and Houston *et al.,* 2012). In the present study, the surfaces of a few stones were cracked like eggshells and revealed numerous calcium phosphates on chemical analysis**.** These findings were in agreement with Suzuki *et al.* 1997, Kruger *et al.* 1999, and Kumar and Srikanth 2021. Males, small breeds, and older dogs are more predisposed to calcium oxalate uroliths. Labrador retrievers, Golden retrievers, and German shepherds appear to be at low risk for calcium oxalate uroliths. Although a genetic basis has not been established as a cause of calcium oxalate formation in dogs, differences in mineral metabolism and urine composition may provide an explanation for such breed susceptibility. For example, Miniature Schnauzers urinate significantly less often, and have a lower urine volume, significantly higher urine pH, and significantly higher urinary calcium concentration than Labrador retrievers. Calcium oxalate stone-formers have higher urinary concentrations of calcium and oxalate, but lower concentrations of potassium and phosphorus. Calcium oxalate uroliths are rarely associated with UTI, and these can form at any physiological urine pH. A major risk factor for calcium oxalate uroliths is urinary calcium and oxalate supersaturation. Intestinal hyperabsorption of calcium has been reported in stone-forming miniature schnauzers. Other factors that have been suggested to promote calcium oxalate supersaturation include excess dietary intake of calcium, vitamin D, or vitamin C; disorders contributing to hypercalcemia (e.g., lymphoma, primary hyperparathyroidism) or calcium mobilization (hyperadrenocorticism, chronic glucocorticoid treatment); and diets containing large quantities of oxalate (spinach, wheat germ, sweet potatoes). Defective nephrocalcin or other natural inhibitors of calcium oxalate uroliths have also been proposed. In epidemiological studies, dry diets especially those resulting in increased acidification of urine have been associated with a greater risk for calcium oxalate uroliths. Dietary factors associated with a decreased risk of calcium oxalate urolithiasis in dogs include increased dietary water, protein, calcium, phosphorus, magnesium, sodium, potassium, and chloride and decreased carbohydrate content (Ling *et al.*, 2001b; Stevenson and markwell, 2001 and Houston *et al.*, 2004). In our study the calcium oxalate monohydrate uroliths are noticed on SEM as picket fence appearance and calcium oxalate monohydrate small crystals. Where as, calcium oxalate dihydrate crystals were retractile, bipyramidal shape and porosity on scanning electron microscopy. These findings were in agreement with Domingo-Neumann *et al.* (1996), Suzuki *et al.* (1997), Sravanthi *et al.* (2014b), Walaa *et al.* (2014), Tufani *et al.* (2017) and Kumar and Srikanth (2021).

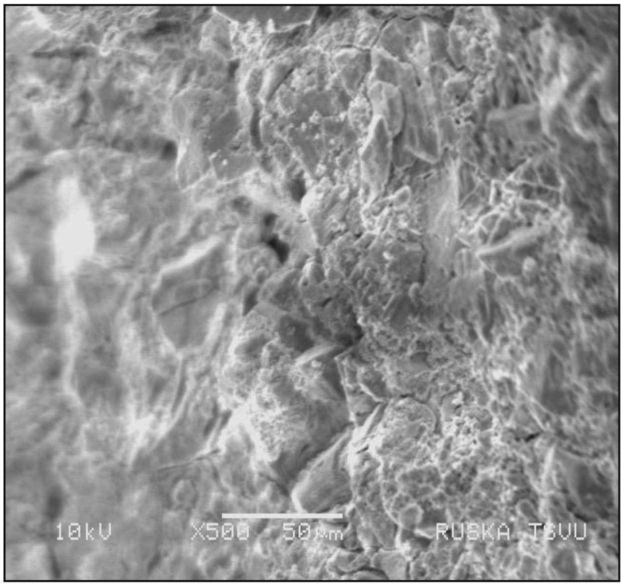

Fig. 1: Scanning electron micrograph showing concentric laminations composed of compact and loosely packed strata- Struvite.

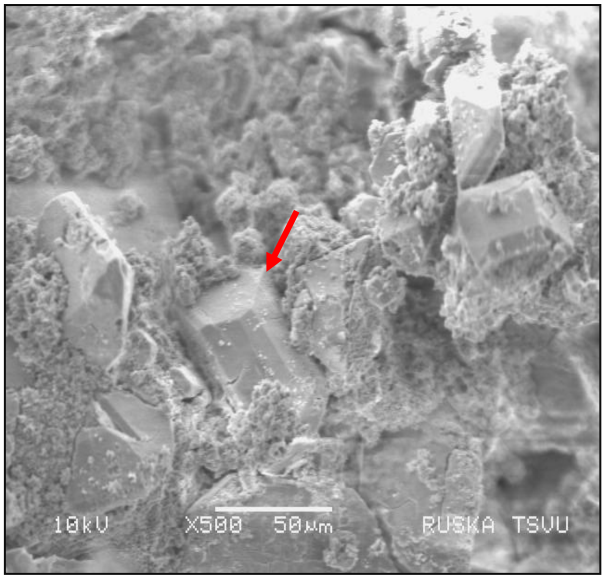

Fig.2: Scanning electron micrograph showing coffin lid shaped crystals at center- Struvite.

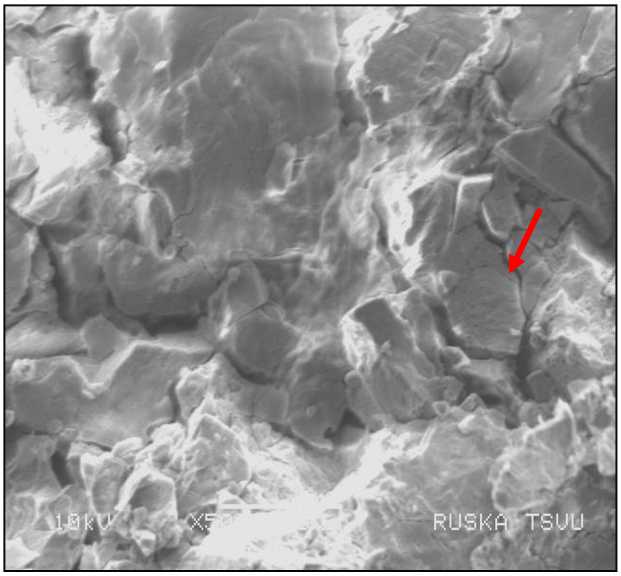

Fig. 3: Scanning electron micrograph showing hexa-hedral coffin lid shaped crystals towards periphery-Struvite.

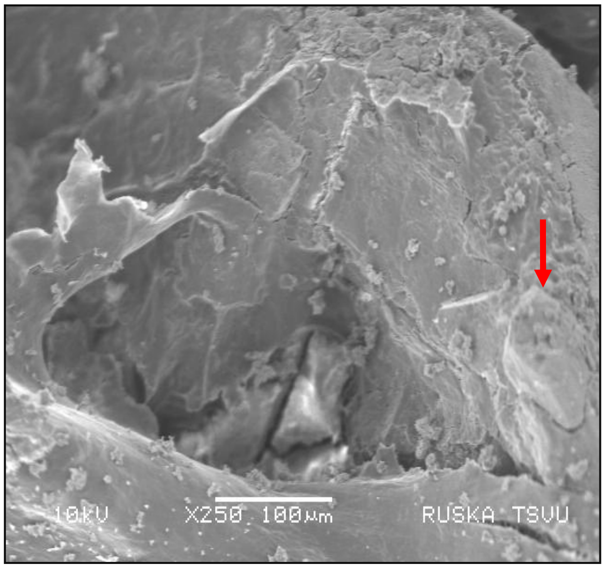

Fig.4: Scanning electron micrograph showing hexa-hedral coffin lid shaped crystals at periphery-Struvite.

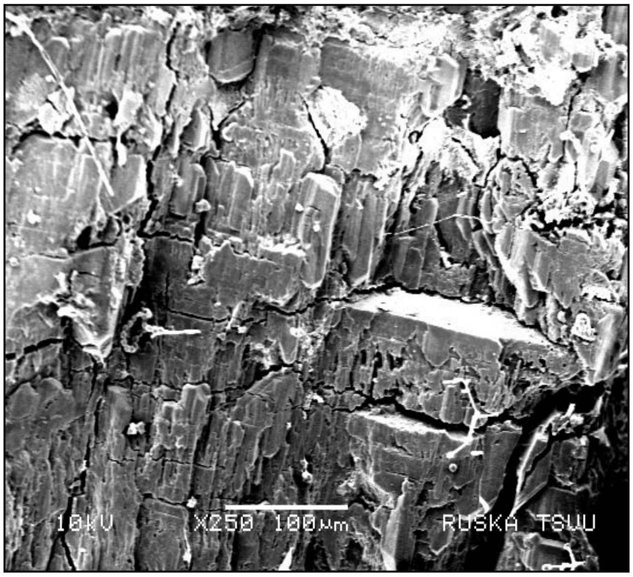

Fig.5: Scanning electron micrograph showing irregularly arranged rock like large magnesium ammonium phosphate crystals.

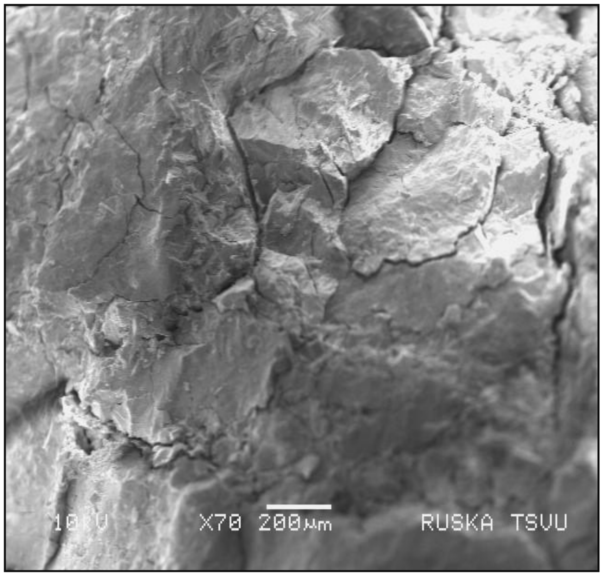

Fig.6: Scanning electron micrograph showing irregularly arranged rock like large magnesium ammonium phosphate crystals.

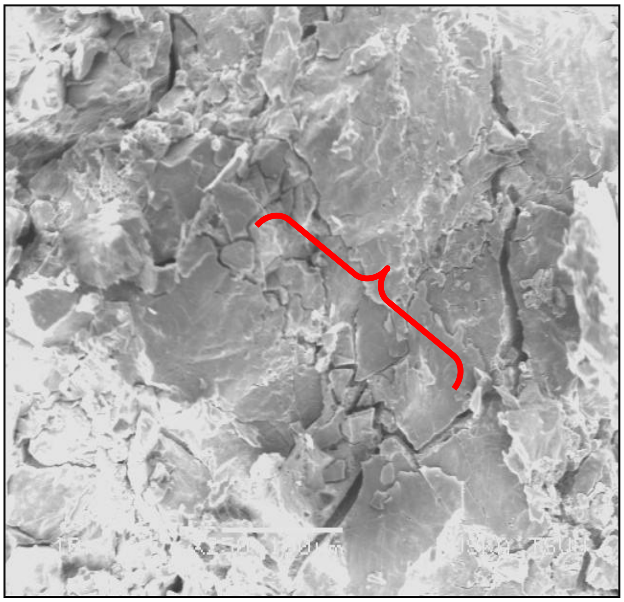

Fig. 7: Scanning electron micrograph showing small sized irregular magnesium ammonium phosphate crystals.

Fig.8: Scanningelectron micrograph showing large sized regular magnesium ammonium phosphate crystals

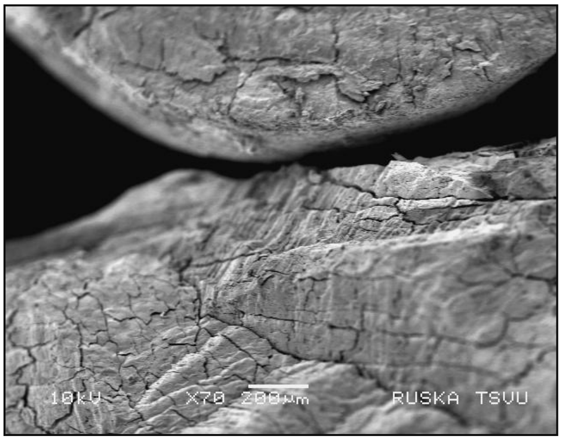

Fig.9: Scanning electron micrograph showing Cracked like egg shells of calcium phosphate calculi surface.

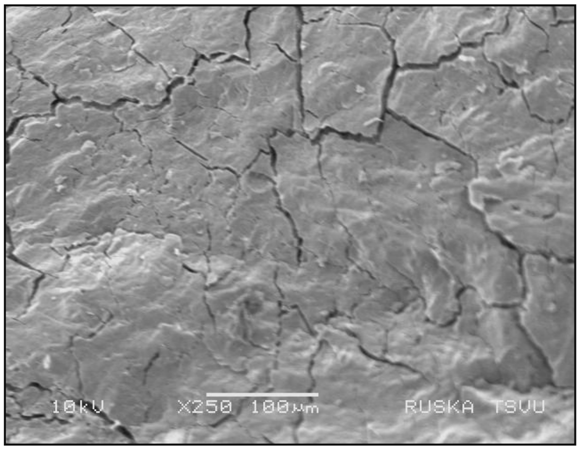

Fig.10: Scanning electron micrograph showing Cracked like egg shells of calcium phosphate calculi surface.

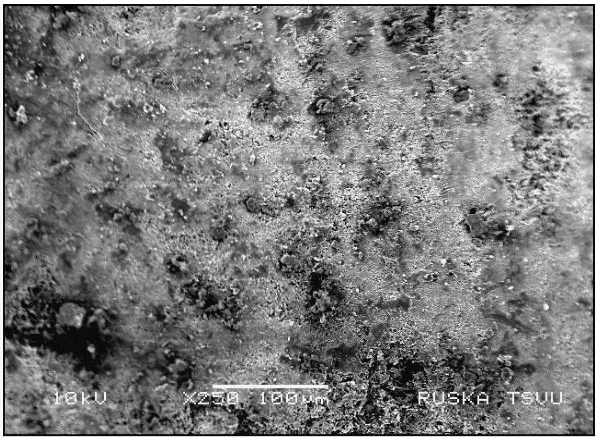

Fig.11: Scanning electron micrograph showing Picket fence appearance of calcium oxalate monohydare crystals.

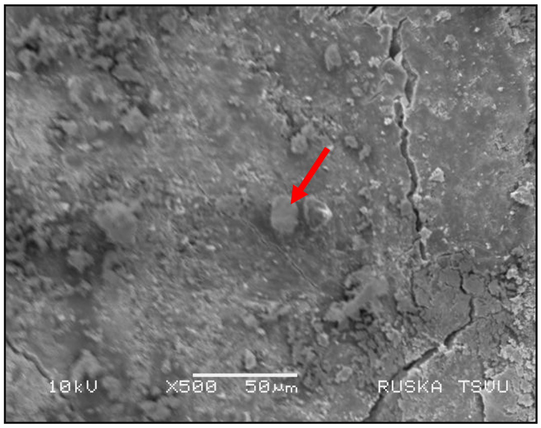

Fig.12: Scanning electron micrograph showing calcium oxalate monohydare crystals.

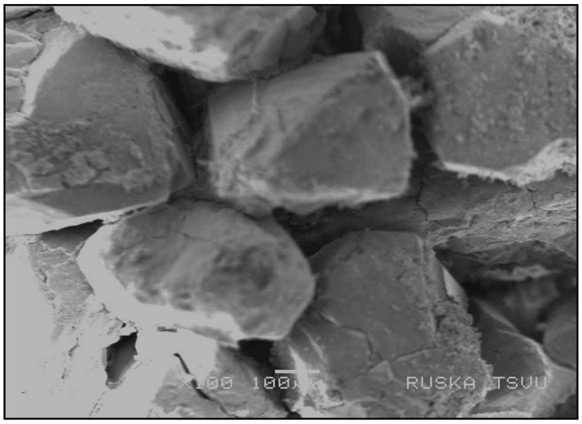

Fig.13: Scanning electron micrograph showing bipyramidal crystals of calcium oxalate dihydrate.

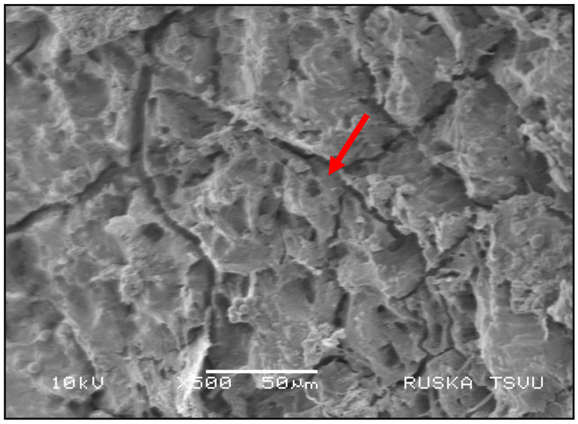

Fig.14: Scanning electron micrograph showing calcium oxalate dihydrate crystals (note the porocity in the caliculi)

5. Conclusion

Quantitative analysis studies like electron microscopy gives good ultra structural characteristics aspects of caliculi and also provides relative percentage composition of each mineral type is preferred over qualitative analysis.

6. Acknowledgment

The authors are thankful to PVNR TVU, Rajendranagar, Hyderabad for providing necessary facilities in completion of research work.

7. References

Fazili M R and M M Ansari. 2007. Prevalence of bovine obstructive urolithiasis in Kashmir valley. Indian Veterinary Journal 84: 540-541.

Kim C W, Choi U S, chegal J, Bae B K, Lee D H, Ko Y H and Lee C W. 2004. Canine Urolithiasis: Interrelation between Breed, Age, Sex, Anatomic Location, Urine pH, Crystal and Mineral Composition of Uroliths (270 cases). *Journal of Veterinary Clinics* **21** (3): 264-269.

Bartges J W and Lane I F. 2003. Medical treatment of urolithiasis. In*: Textbook of Small Animal Surgery* **3** (2): 1661-1671.

John J B and Lonnie D R. 1998. In: *Electron Microscopy Principles and Techniques for Biologists.*

Lakshman M. 2017. Diagnostic Electron Microscopy (EM) for Avian Diseases-An Overview. *International Journal of Science and Research* **6** (5): 1478-1483.

Lakshman M. 2019. Application of conventional electron microscopy in aquatic animal disease diagnosis: A review *Journal of Entomology and Zoology Studies* **7** (1): 470-75.

Shaw D H and L I Sherri, 1997. Urinary tract diseases and Fluid and electrolyte disorders. In: Small Animal Internal Medicine I ed. Williams and Wilkins, USA, P:113.

Suzuki T, Yano M, Sumi S, Honda M, Hosoya Y and Yoshida K I. 1997. Study of the structure of struvite stones with scanning electron microscopy and energy-dispersive X-ray microanalysis. *Urologia internationalis*, **58** (2), 88-92.

Sravanthi P, Sekhar E L C, Raghavender K B P and Kumar D P. 2014b. Studies on electron microscopy of uroliths in dogs. *International Journal of Veterinary Science*, **3** (4), 177-180.

1Kumar K S and Srikanth K. 2021. Scanning Electron Microscopic Structure and Composition of Urinary Calculi of Geriatric Dogs. *International Journal of Veterinary Science and Animal Husbandry* **7** (1): 081-087.

Lulich J P, Osborne C A, Bartges J W and Lekcharoensuk C. 2000. Canine lower urinary tract disorders. *Textbook of Veterinary Internal Medicine* **2**: 1805-1832.

Houston D M, Moore A E, Mendonca S Z and Taylor J A. 2012. 2, 8-dihydroxyadenine uroliths in a dog. *Journal of the American Veterinary Medical Association*, **241** (10): 1348-1352.

Kruger J M, Osborne C A and Lulich J P. 1999. Canine calcium phosphate uroliths: etiopathogenesis, diagnosis and management. *Veterinary Clinics: Small Animal Practice*, **29** (1), 141-159.

Ling G V, Thurmond M C and Choi Y K. 2001b. Changes in proportion of canine urinary calculi composed of calcium oxalate or struvite in specimens analyzed from 1981 through 2001. *Journal of Veterinary Internal Medicine*. **17**:817-823.

Stevenson A E and Markwell P J. 2001. Comparison of urine composition of healthy Labrador retrievers and miniature schnauzers. *American Journal of Veterinary Research* **62**: 1782–86.

Houston D M, Moore A E, Favrin M G and Hoff B. 2004. Canine urolithiasis: a look at over 16000urolith submissions to the Canadian Veterinary Urolith Centre from February 1998 to April 2003. *The Canadian veterinary journal* **45** (3): 225-230.

Domingo-Neumann R A, Ruby A L, Ling G V, Schiffman P S and Johnson D L. 1996. Ultrastructure of selected struvite-containing urinary calculi from dogs. *American journal of veterinary research*, **57** (9), 1274-1287.

Walaa I, Mohamaden H, Wang H, Guan M and Xia J L. 2014. Electron imaging of calcium oxalate crystals in beagle dogs' urine. *International Journal of Veterinary Science and Medicine* **2**: 83-88

Tufani N A, Singh J L, Kumar M and Rajora V S. 2017. Diagnostic evaluation of renal failure in canine with special reference to urinalysis. *Journal of Entomology and Zoology Studies* **5** (6): 2354-2364.