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Efficacy of Fourier Transform Infrared Spectroscopy for the Analysis of Urinary Stones in Canines

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ABSTRACT:The study was carried out to assess different methods for the identification of mineral components in uroliths in dogs presented to different hospitals under Kerala Veterinary and Animal Sciences University. Dogs with clinical signs of urolithiasis like stranguria, dysuria, haematuria and dribbling of urine were screened and 12 dogs were confirmed to have urolithiasis using clinical examination, imaging techniques and urinalysis. The stones were surgically retrieved from the bladder or urethra depending upon the site of obstruction. After retrieval, the stones were powdered and viewed under light microscope for morphological interpretation. The mineral components were subjected to semi-quantitative colorimetric detection by means of a urinary stone analysis kit. Fourier transform infrared (FT-IR) spectroscopy was also used to determine the mineral component of stones. Among the different techniques, FT-IR was the most effective and time saving method to detect minor and major components present in the urinary stones within a short time. The present study advocates the use of Fourier Transform Infrared Spectrophotometer for the identification of urinary stones in the management of urolithiasis.

KEYWORDS: Urolithiasis ,dogs, FT-IR

I. INTRODUCTION

The urinary system is designed to dispose the waste in a liquid form (Spencer, 2016). Urine, the liquid byproduct of urinary system contains inorganic and organic minerals which are in balance. When there is super saturation, the constituents can precipitate and aggregate forming solid concretions known as urinary stones. Urolithiasis is a recurrent clinical problem in dogs throughout the world (Vrabelova *et al.*, 2011). The two most common mineral types reported in dogs are struvite (magnesium ammonium phosphate hexahydrate) and calcium oxalate. Analysis of mineral constituents is necessary for formulating an effective treatment and management protocol. This study aims to evaluate the efficacy of different techniques for identifying the mineral components present in urinary stones.

II. RELATED WORK

Many research work have been conducted on the comparison of different diagnostic techniques for the analysis of mineral composition of urinary stones in canines. The efficacy of FT-IR in the analysis of urinary stones have been reported by few authors.

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III MATERIALS AND METHODS

The different methods employed for identifying the mineral status of uroliths and procedures are detailed as follows.

2.1. Urine sediment examination

About five milliliters of urine was collected through catheterization and one drop of urine was transferred onto a glass slide and examined under the high power objective of the microscope. The remaining urine was transferred into a graduated centrifuge tube and centrifuged at 1500 rpm for five minutes. The supernatant was discarded and the sediment was examined under high power to detect the presence of crystals. The examination was completed within 30 minutes to avoid the precipitation of crystals (Plate 1).

2.2 Microscopic examination of stone

The retrieved stones were thoroughly washed, dried, powdered and examined under the microscope. The shape, size and texture was recorded (Plate 2a,2b).

2.3 Chemical method (Semi-quantitative colorimetric detection)

The different mineral components were analyzed using a semi quantitative kidney stone analysis kit (ELITEch Group). Different reagents were used for detection of different minerals by colorimetry. The colour reaction was compared to the standard colour limits. The percentage of the mineral component was calculated by multiplication with a preordained factor. The observations were recorded (plate 3a,3b,3c,3d,3e).

2.4. Fourier Transform Infrared Spectrophotometer

The retrieved stones were washed, dried overnight at 37 °C and powdered. To create a background blank in the machine, 300 mg of potassium bromide (KBr) was pressed under pressure to form a pellet and analysed. Subsequently, 298 mg of KBr and 2 mg of powdered uroliths were mixed thoroughly. It was then pressed under pressure and the pellet was scanned and analysed to record the spectrum. The spectrum was compared to determine the mineral components.

LIST OF PLATES

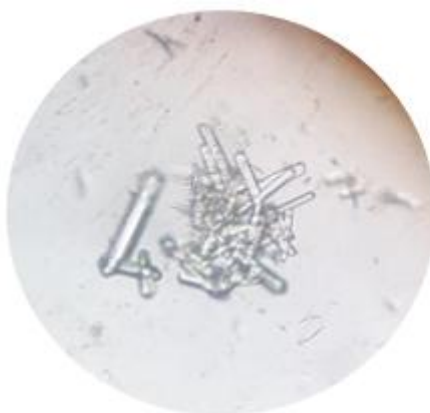


Plate 1- Struvite crystal- 10 x

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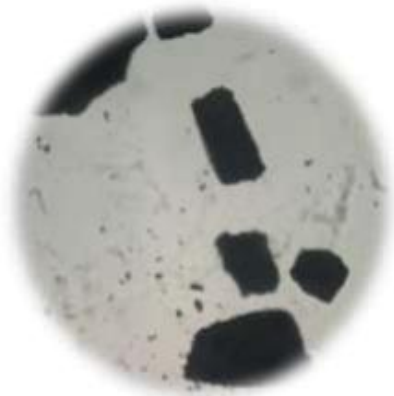


Plate 2a- Microscopic examination
of stone-10x

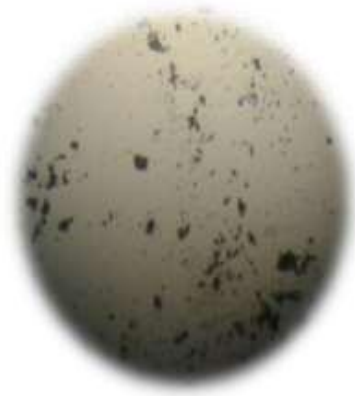


Plate 2b- Microscopic examination
of stone-10x



Plate3a-
Magnesium
positive



Plate3b-
Ammonium
positive



Plate 3c-
Phosphatepo
sitive



Plate 3d-
Calcium
Positive



Plate 3e-
oxalate
positive,

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IV. RESULTS

iv.i. Fourier Transform Infrared Spectrophotometer

This study showed that 58.33 percent of the stones (7 out of 12 cases) were a combination of struvite, calcium oxalate dihydrate and carbonate apatite whereas 41.66 percent were a combination of calcium oxalate monohydrate and calcium phosphate.

iv.ii. Urine sediment examination

Crystals were (struvite) in one sample out of the 12 samples (8.33 per cent).

iv.iii. Microscopic examination of urolith

All the stones when viewed under light microscope did not show much difference in morphology.

iv.iv. Chemical method

Minerals such as calcium, magnesium, phosphate and ammonium were observed whereas it was not sensitive for the detection of oxalate.

Table 1- Sensitivity of kit method in detecting different mineral types

Sl.No.	Mineral type	No. of presented cases	No. of positives detected	% sensitivity
1	Magnesium	7	7	100
2	Ammonium	7	7	100
3	Phosphate	12	12	100
4	Calcium	12	12	100
5	Oxalate	12	variable	Nil

V. DISCUSSION

Among the different techniques employed to identify the mineral composition of stones, infrared spectroscopy was the most sensitive method as it could detect a combination of minerals. According to Langston *et al.* (2008), crystalluria is insensitive and unreliable because crystals associated with a particular stone disappears from the urine when the stones form in the urinary tract. Wet method is time consuming as well as ineffective in determining the percentage of the minerals as well as stones like silica. Analysis of the stones for determining the morphology is not practical as the uroliths are made up of many molecules in continuous bonding and powdering would destroy the ultrastructure. Most of the stones are made up of a core and a matrix. Infrared spectroscopy detects the major component in a group of components thereby enabling the observer to distinguish between the core and the matrix. According to Manning and Blaney (1986), infra-red spectroscopy has many advantages over conventional methods for identification of all components which are even in trace quantities that are likely to be found in uroliths of domestic animals. Hence, this technique can identify crystalline and amorphous minerals even if present at low concentration and quantity and is useful for all types of stones (Vergauwe *et al.* 1994).

VI. CONCLUSION

From the present study, it can be concluded that Fourier Transform Infrared Spectrophotometer was efficient in diagnosing a variety of mineral components compared to other techniques.

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