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Fourier transform infrared spectrophotometer with attenuated total reflectance (FTIR-ATR) for analysis of uroliths in caprine species

Anvitha Hansoge[1], Sudheesh S Nair[2], M.V. Chinnu[3], M.K. Narayanan[4] and K.D. John Martin[5].
Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala Veterinary and Animal Sciences University, India

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Abstract

Pathophysiology of obstructive urolithiasis in goats are multifactorial involving management, nutritional and hormonal factors. Analysis of the composition of a particular urolith would aid in understanding the aetiopathogenesis of the calculi which can facilitate establishment of appropriate treatment and prevention protocols. Four cases of chronic obstructive urolithiasis in male goats presented to University Veterinary Hospital were surgically managed by tube cystotomy. The calculi extracted from the bladder were subjected to Fourier transform infrared spectrophotometer with attenuated total reflectance (FTIR-ATR) to identify the composition of the calculi. The infrared wavelength bandwidths of H-O-H stretching vibrations, H-O-H bending modes of vibrations of water molecules, N-H symmetric stretching vibrations, N-H bending vibration and N-H asymmetric bending vibration in NH4+ unit's ionic phosphate were compared with the available reference spectrum of wavelength and were found to be identical and comparable with the standard infrared wavelength of struvite calculi.

Key words: *Fourier transform infrared spectrophotometer, Attenuated total reflectance, Chronic obstructive urolithiasis, Goats*

Urolithiasis is a clinical condition resulting from multiple congenital and/or acquired pathophysiologic processes that result in an elevated concentration of insoluble crystalloids in the urine (Dar, 2011). Pathophysiology of obstructive urolithiasis were identified to be multifactorial

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1. *M.V.Sc. student email : anvi.hansoge@gmail.com Ph. 9590125001*
2. *Assistant Professor*
3. *Assistant Professor, Department of Veterinary Biochemistry*
4. *Professor*
5. *Professor and Head*

involving management, nutritional and hormonal factors. Sun *et al.* (2010) reported the most common aetiology of formation of urolithiasis as the supersaturation of the excretory solutes in the urine, increased concentrate feeding and lack of sufficient water intake and that the analysis of the composition of a particular urolith would aid in understanding the aetiopathogenesis of the calculi and further facilitated establishment of appropriate treatment and prevention protocols. Primiano *et al*. (2014) reported that FT-IR was found to be the gold standard test for analysis of urinary calculi and that it showed a high sensitivity thereby giving an accurate recognition of the composition of each stone.

Materials and Methods

Four male goats with chronic obstructive urolithiasis with an intact bladder were presented to University Veterinary Hospital, Mannuthy with history of stranguria. All animals had the history of being fed with high concentrate diet (cattle ration). The Ultrasonography evaluation revealed hypoechoic turgid bladder with hyper echoic uroliths forming sludge in the bladder floor that made wavy motion on gentle succession. (Fig-1) The animals underwent tube cystostomy by standard procedure documented by Gazi *et al.* (2014). The urinary calculi was collected by sieving the urine (Fig-2) The microanalysis of the sediments revealed uroliths similar to struvite crystals. (Fig- 3)

The urolith samples were serially numbered as A_1, A_2, A3 and A4 respectively. The calculi were thoroughly washed with deionized water to remove the adhered dirt and blood clots, dried at 37°C and stored for analysis of the mineral content. Analysis of calculi was done using Perkin Elmer Spectrum 2 Infra-red Spectrophotometer - USA using the principle of Fourier transform infrared spectrophotometer with Attenuated Total Reflectance (FTIR-ATR) at Central Instrumentation Laboratory, College of Veterinary and Animal Sciences, Mannuthy. To create a background blank in the machine, 300 mg of potassium bromide (KBr) was pressed under pressure to form a pellet and analysed. (Fig-3) Subsequently, 298 mg of KBr and 2 mg of powdered uroliths were mixed and was pressed under pressure and the pellet was scanned and analysed to record the spectrum using FTIR- ATR machine and the observed spectrum was compared to the standard reference spectrum based on the observed transmittance and frequency of the waveforms.

Results and Discussion

The results of analysis of calculi in Fourier Transform Infrared Spectrometry with Attenuated Total Reflectance (FTIR-ATR) are depicted in Table 1. Analysis of the calculi from animal no. A_1, A_2, A3 and A4 using FTIR-ATR revealed a graph with an IR spectrum ranging from 4000 to 450 cm^{-1}. The H-O-H stretching vibrations of water of crystallization were at 3401.94 cm^{-1} for animal no A_1, 3391.81 cm^{-1} for animal no A_2, 3500 to 3350 cm^{-1} for animal no A_3 and 3360.29 cm^{-1} for animal no A_4. The H-O-H stretching vibrations of the cluster of water molecules were at 2231.52, 2346.67 and 2321.69 cm^{-1} for animals' A_1 A_2 A_3 A_4 respectively. The H-O-H bending modes of vibrations ranged from 1440.19, 1434.90, 1434.68 and 1441.17 cm^{-1} for animals' A_1 A_2 A_3 A_4 respectively. The N-H symmetric stretching vibrations were at 3401.94 cm^{-1} for animal no A_1, 3391.81 cm^{-1} for animal no A_2, 3500 to 3350 cm^{-1} for animal no A_3 and 3360.29 cm^{-1} for animal no A_4. The N-H bending vibration was at 1670.28, 1650.70, 1651.8 and 1654.67 cm^{-1} for animals' A_1 A_2 A_3 A_4 respectively and the N-H asymmetric bending vibration in NH4+ unit's ionic phosphate was at 995.16, 1000.4, 1000.48 and 1000.14 cm^{-1} for animals A1 A2 A3 A4 respectively. These infrared wavelength bandwidths were compared with the available reference spectrum of standard wavelength of struvite calculi proposed by Bindhu and Thambi (2012). The infrared spectrum from animals are depicted in the figure 5-8.

The spectra contained a bandwidth comparable to that of the standard IR spectra of struvite crystals as proposed by Bindhu and Thambi (2012) who reported that in a pure magnesium ammonium phosphate spectrum, the broad envelop at 3270 cm^{-1} correlates to the O-H and N-H stretching vibrations. The band at 2935 cm^{-1} is due to NH4 + ion and the

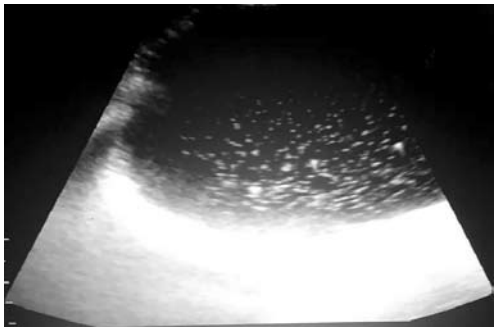

Fig-1 Ultrasonography to detect uroliths

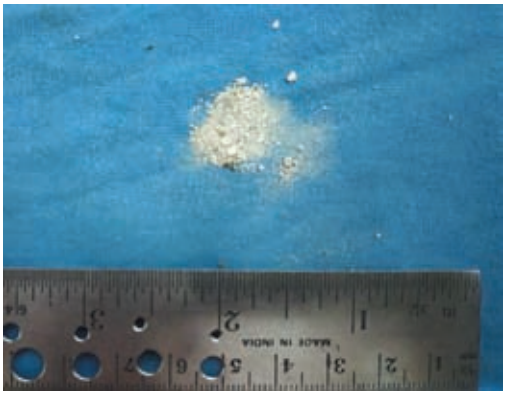

Fig-2 Uroliths retrieved from urinary bladder by tube cystostomy

Fig-3 Microscopic examination of struvute cystals

band corresponding to 1666 cm^{-1} was assigned to N-H bending vibrations. The sharp yet weak envelop correlating to 1445 cm^{-1} could be due to N-O asymmetric stretching vibration. The absorption band occurring at 1010 cm^{-1} was assigned to ionic phosphate.

Fig-3 Prepared pellets of KBr and urolith for FTIR – ATR spectroscopy

Fig-4 Perkin Elmer Spectrum 2 Infra-red Spectrophotometer- USA

The determination of molecular composition, crystalline composition and the quantification of all stone components are helpful to establish the etiology of stones disease and consequently aid in the treatment of the clinical disease. Various methodologies exist for stone analysis. Fourier transform infrared spectroscopy (FT-IR) is becoming the gold standard for stone analysis as it is the most appropriate technique. The principle of FT-IR involves generating an infrared spectrum from the vibrational motion of the molecules. These vibrational frequencies are unique for each compound. This feature of the spectrometer is used to characterize inorganic compounds from organic ones present in the calculi. The band intensities are proportional to the compound concentration and hence qualitative estimations are also obtained. FT-IR spectroscopy leads to unambiguous information about the stone composition, both for main substances and trace elements, all essentials to guide therapy.

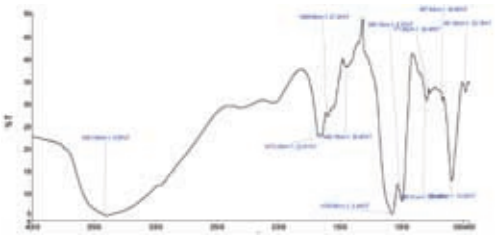

Figure 5: Infrared spectrum from goat A_1 showing struvite bands at 3401, 1440, 1670 and 995.16 cm^{-1}

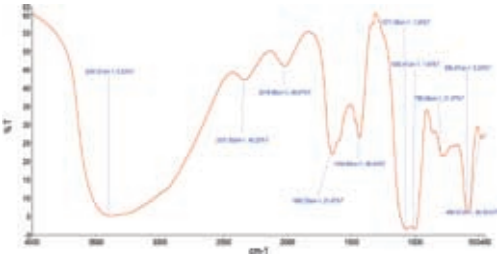

Figure 6: Infrared spectrum from goat A_2 showing struvite bands at 3391, 1434, 2331, 1650 and 1000.4 cm^{-1}

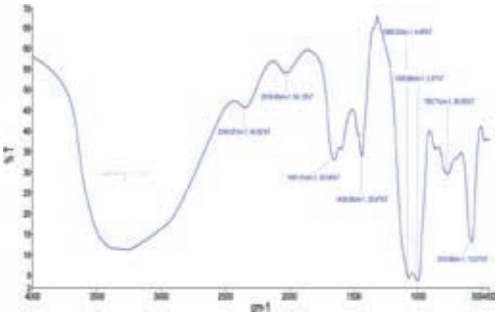

Figure 7: Infrared spectrum from goat A_3 showing struvite bands at 3500-3350, 1434, 2346, 1and 1000.48 cm^{-1}

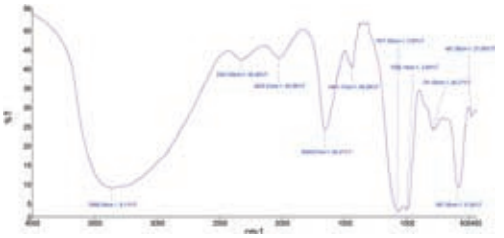

Figure 8: Infrared spectrum of goat A_4 showing struvite bands at 3360, 1441, 2321, 1654 and 1000.14 cm^{-1}

Wang *et al*. (2009) explained the preparation of the calculi before spectroscopy by washing the crystals twice in deionising water and air drying overnight. The authors recorded strong bands of wavelength 556.1, 986.4, 1066.7, 3201.7 cm^{-1} and medium-strong bands at 1673.9, 2385.2 cm^{-1} which was conclusive of magnesium ammonium phosphate crystals. Bindhu and Thambi (2012), in their study on microanalysis of the struvite crystal, reported that the standard IR spectra ranged within 4000 to 450 cm^{-1} for pure struvite with the broad envelop at 3270 correlated to the O-H and N-H stretching vibrations. In their study, the band at 2935 cm-1 was due to NH4 + ion and the band corresponding to 1666 cm-1 was assigned to N-H bending vibrations. The sharp yet weak envelop of 1445 cm-1 was due to N-O asymmetric stretching vibration. The absorption band occurred at 1010 cm-1 was assigned to ionic phosphate.

In the present study based on the alkaline pH of urine in all animals and the confirmed composition of struvite, medical management was done with ammonium chloride at 300 mg/kg body weight per orally and the owners were advised to avoid feeding cattle feed to goats and provide ad libitum water.

Conclusion

Urolithiasis is a clinical disease affecting the feedlot animals fed on high concentrate diet (cattle ration). The most common urolith manifested in male goats is magnesium ammonium phosphate (struvite) calculi. The use of Fourier transform infrared spectroscopy has been highly specific for determining the composition of the urolith which invariably has aided in specific treatment protocols and preventive management strategies in caprine chronic obstructive urolithiasis.

Acknowledgement

Central Instrument Laboratory, CVAS, Mannuthy.

Table 1. Results of analysis of calculi in Fourier Transform Infrared Spectrometry with Attenuated Total Reflectance (FTIR-ATR)

Functional Group Assignments	Animal no.	Reported IR wavelength (cm^{-1})	Standard IR wavelength of pure struvite (cm^{-1}) (Bindhu and Thambi, *2012)*
H-O-H stretching vibrations of water of crystallization	A_1	3401.94	3270
	A_2	3391.81	
	A_3	3500-3350	
	A_4	3360.29	
H-O-H stretching vibrations of a cluster of water molecules	A_1	-	2385
	A_2	2331.52	
	A_3	2346.67	
	A_4	2321.69	
H-O-H bending modes of vibrations	A_1	1440.19	1445
	A_2	1434.90	
	A_3	1434.68	
	A_4	1441.17	
N-H symmetric stretching vibrations in NH4+ units		-	2935
N-H symmetric stretching vibrations	A_1	3401.94	3270
	A_2	3391.81	
	A_3	3500-3350	
	A_4	3360.29	
N-H symmetric stretching vibrations in NH4+ units		-	2935
N-H bending vibration	A_1	1670.28	1666
	A_2	1650.70	
	A_3	1651.8	
	A_4	1654.67	
N-H asymmetric bending vibration in NH4+ units Ionic phosphate	A_1	995.16	1010
	A_2	1000.4	
	A_3	1000.48	
	A_4	1000.14	

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