Struvite Stones and the Microbiome

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UCDAVIS VETERINARY MEDICINE
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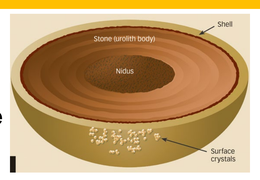
Introduction

Enteroliths in Horses and Struvite Uroliths in Dogs and Cats

Endogenously produced mineral concretions, form in concentric rings around a foreign object (nidus). **Enteroliths occur in the right dorsal colon of horses while uroliths occur along the urinary tract of dogs and cats.**

Primary component= Struvite

- A composite of **magnesium ammonium phosphate hexahydrate [(NH4)MgPO4•6H2O)]**
- **Alkaline pH is favorable for precipitation**
- Struvite uroliths are the **most common type of urinary stone in dogs, 2nd most common type in cats.**



https://www.cliniciansbrief.com/article/urolithiasis-diagnostic-imaging-clinical-signs

In dogs, virtually all struvite uroliths are infection induced.

Most common types of bacteria:

1. *Staphylococcus psuedintermedius*
2. *Proteus mirabilis*
3. *Klebsiella* spp. (known N fixer)

Two ways to produce NH_3:

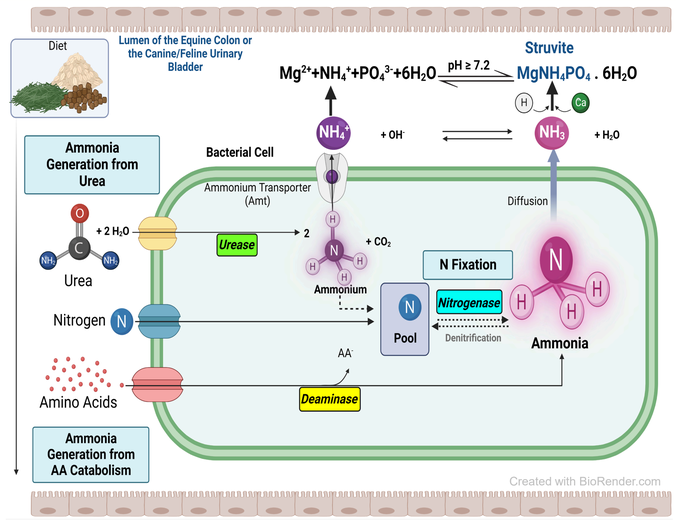
Urease producers- *Staph.* and *Proteus*

Associated with UTIs and struvite formation

Urease → hydrolysis of urea, forming NH_3 and CO_2.

N Fixers-*Kelbsiella*

Nitrogenase → microbial production of NH_3



Purpose

- **Urolithiasis occurs in a vast array of species**
- **Primary stones that occur in dogs and cats are struvite and CaOx**
- Struvite is the common denominator, but **stone characteristics have not been compared at depth**
- **Insight into the disease processes can be used in the clinical setting, optimizing treatment options**
- Methods **can be applied to other types of urinary stones.**
- **Translational applications exist for both humans and many veterinary species.**

Objectives

Hypotheses:

1. A microbial community exists **(microbiome) within struvite urinary stones in both dogs and cats that contributes to stone formation.**
2. Based on previous findings, **we postulate that a similar microbiome occurs in equine enteroliths and dog/cat struvite urinary stones. Members of the microbiomes contribute to an environment that favors struvite precipitation and stone formation.**

Objective: Identify the microbiome of canine/feline struvite urinary stones **via deep RNA metagenomic sequencing.**

Methods

Sample Selection

Samples selected using the stone database of the Gerald V. Ling Urinary Stone Analysis Laboratory at UC Davis. For each sample, the mineral composition of the layers and the core had been determined by the lab using optical crystallography with polarizing light microscopy.

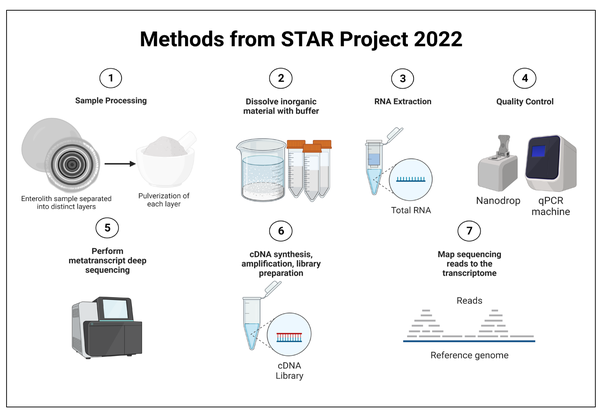
Mineral composition was displayed as seen here:

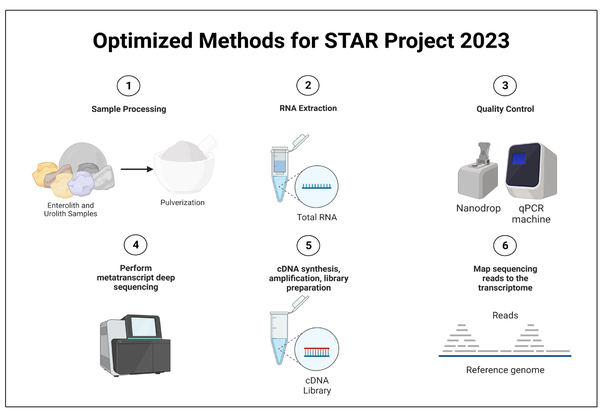
SPECIES	COMP_OUTR	LAYER_1	LAYER_2	LAYER_3	CORE
CANINE	struvite 100%	struvite 95%, apatite 5%	struvite 99%, apatite 1%	-	struvite 100%

Samples were selected from the lab's database based on the following criteria:

1. Species (canine, feline, or equine)
2. Urine culture performed
3. Stone culture performed
4. Layers and the core composed primarily of struvite (some carbonate apatite was acceptable)

Results: RNA Extraction Optimization

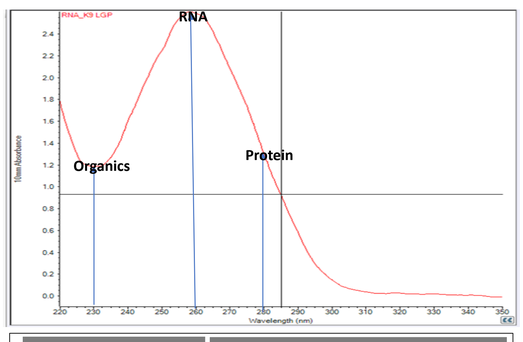




By optimizing the methods used for RNA extraction we were able to increase both the concentration and the quality of the RNA extracted.

- Eliminating step 2 preserves more sample RNA.
- The acidic buffer likely degraded some of the RNA previously.
- Pulverizing the stones was effective for quality RNA extraction.
- Each sample was crushed whole to detect the presence or absence of RNA within each stone.
- Separating different urolith layers by hand is not feasible.

Results: RNA Extraction from Enteroliths and Uroliths



Compound	Nanodrop Absorbance Wavelength (nm)
RNA	260
Proteins	280
Organics	230

260/280= how "pure" a sample is from protein, ideal ~2

260/230= how "pure" a sample is from organic contaminants, ideal ~2

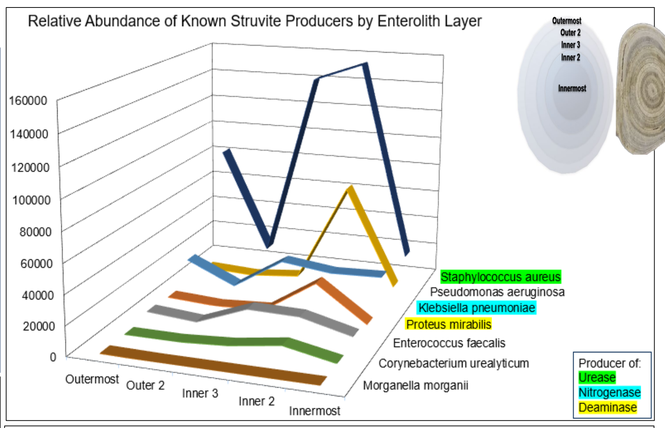
Nanodrop Results by Species

	RNA Conc. (ng/µl)	Protein (260/280)	Organics (260/230)
Canine Urolith	103.5	1.98	2.19
Feline Urolith	201.0	1.85	2.04
Equine Enterolith	54.4	1.62	0.50

Comparison of Enterolith RNA Extraction Methods

Enterolith Sample	RNA Conc. (ng/µl)	Protein (260/280)	Organics (260/230)
Processed with buffer (2022) 	27.7	1.50	0.10
Processed without buffer (2023) 	54.4	1.62	0.50

The optimized extraction methods were found to produce an RNA sample of higher concentration and quality for deep sequencing.



These organisms have been identified as contributors to struvite stone formation in vivo. *Staphylococcus aureus* is the most common *Staph.* species contracted by horses. Similarly, *Staphylococcus psuedintermedius* is the species most common in dogs and cats. *Proteus mirabilis* and *Klebsiella* spp. listed here, are the 2nd and 3rd most common urease producers associated with canine struvite uroliths.

Conclusion and Future Directions

Future Directions :
Deep RNA metagenomic sequencing is currently underway for the canine and feline urolith samples along with more enterolith samples.

1. Determine the relative abundance of struvite producers in both the canine and feline uroliths and compare to enterolith data.
2. Work to determine function using transcripts from the metagenome The transcripts allow identification of genes associated urease, but also broader NH_3 and CO_2 metabolism associated with struvite formation.
3. Apply these techniques to other types of uroliths

Conclusions:

1. An **active microbiome is present in enteroliths and canine/feline struvite uroliths**
2. Enteroliths and struvite uroliths **contain quality, extractable RNA**
3. The **sample preparation is a key aspect** of analyzing these sample types
4. The stones **contain a microbial community that likely helps facilitate struvite formation both in the equine GI tract and the canine/feline urinary systems**

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