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DEDICATION

To my parents: Ezinna Lawrence Nwaokorie Okoroafor and Ezinne Lydia Oyiridiya Nwanyianyanwu Okoroafor

For their love for knowledge through education
ABSTRACT

Objective--To test the hypothesis that the predominant mineral type in naturally occurring ferret uroliths was sterile struvite and to determine if age, breed, gender, reproductive status, geographic location of ferrets, season of the year of uroliths submission, and anatomic location within the urinary tract were risk factors associated with ferret sterile struvite uroliths formation.

Design--Case-control retrospective study

Animals--408 case ferrets from the Minnesota Urolith Center (MUC) and 6528 control ferrets from the Veterinary Medical Data Base (VMDB) 1981 through 2007.

Procedure-Historical information about age, gender, reproductive status, anatomic location within the urinary tract, and season of the year of urolith submission were obtained about each ferret. The association between these factors and outcome (sterile struvite urolith formation) was statistically assessed.

Results--Sterile struvite was the predominant mineral in ferret uroliths. Cystine comprised the next most common type of urolith, followed by calcium oxalate. Neutered male ferrets had increased risk of developing sterile struvite uroliths. A significant association was also found between the ages of 2 years and < 7 years and the detection of struvite uroliths. Also a significant association was found between advancing age and the detection of struvite uroliths. Ferret struvite uroliths were more likely to be retrieved from the lower urinary tract (bladder and urethra, n = 254) than from the upper the urinary tract (kidney and ureter, n = 4) (the location of 14 uroliths was not recorded).
Conclusion and Clinical Relevance-- Knowledge of predominant mineral type in uroliths along with insight into etiologic, demographic, and environmental risk and protective factors for urolithiasis may facilitate development of surveillance strategies that could result in earlier detection of uroliths in ferrets. Modification of risk factors including dietary risk factors may help to minimize urolith formation, dissolve existing uroliths and minimize urolith recurrence.

In context of sterile struvite urolithiasis, ferrets and domestic cats are remarkably similar. Both species are true carnivores. Both species form sterile struvite uroliths. In both species, infection-induced uroliths are uncommon. In both species, bacterial urinary tract infections are apparently uncommon. The advent of safe and effective diet therapy to induce dissolution of sterile struvite uroliths in cats, and the striking parallels between ferret and feline urolithiasis, prompts the question as to the safety and efficacy of diet-induced dissolution and prevention of sterile struvite urolith formation in ferrets. The primary goal of this study was to compare features of sterile struvite in cats with those in ferrets. Results of these comparisons may provide a logical evidence-based rationale for clinical trials to determine the safety and efficacy of diet-induced dissolution of sterile struvite uroliths in ferrets.
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CHAPTER 1

HISTORY OF FERRETS
CHAPTER 1

HISTORY OF FERRETS

The domestic ferret species (Mustela putorius furo) belongs to the family Mustelidae and genus Mustela. The other members of the family include minks, weasels, otters, skunks, badgers and European polecats. Domestication of ferret is believed to have started in ancient Egypt (North Africa) about 2500 years ago. However, the origin of domesticated ferrets, unlike that of many domesticated species, is still debatable because to date skeletal remains have not been recovered. This is unexpected considering the rich and varied faunal remains and mummies recovered in ancient Egypt.

Ferrets are close relative of polecats. It is still debatable as to whether or not ferrets are a domesticated form of European polecats, (black footed polecats). Domestic ferrets have white, black, brown or mixed hair coats. The males are called “hobs” and the females are called “jills”.

The average length of domestic ferrets is ~20 inches including 5 inches of tail. Adult ferrets weigh about 2 pounds. They have a life span of 7 to 10 years. Ferrets’ heads are triangular in shape, broad between the ears, while tampering towards the muzzle giving them an elliptical shape, which is perfect for their burrowing behavior. They are social animals and are friendly and inquisitive. They make great pets, but require patience to be litter trained.

The name "ferret" was derived from Latin word “furonem” meaning "little thief" or “Thief”. They were given this name because of their tendency to hide toys and
their habit of hoarding food. In the ancient Roman Empire ferrets were used primarily for
vermin control, and to hunt rabbits and rodents. Their long lean bodies and triangular
head create an elliptical shape that allows them to go down holes and chase rodents and
rabbits out of their burrows.

Ferrets share many anatomical and physiological features with humans as a result
they are used as experimental subjects in biomedical research. Subsequently there is great
demand for ferrets as alternatives to dogs, cats and primates for study of human diseases.
For instance ferrets have been used for the following studies: pediatric tracheal
intubation, cardio-pulmonary, gastro-intestinal, ischemia models, ion exchange in heart
muscles, pulmonary mucus secretion in asthma or influenza and neurological changes
associated with brain and spinal cord injury.

Ferrets were not common in the USA until the 1980s when a veterinarian and former folk
singer, Dr Wendy Wintead, imported the first ferret in 1969. She subsequently
introduced them to a number of celebrities. According to a study carried out by the
California State Bird and Mammal Conservative Program, 800,000 or more domestic
ferrets were kept as pets in the USA as of 1996. In the USA, ferrets are now ranked as
one of the most popular household pets after cats and dogs. They are very often exhibited
in zoos.

Ferrets are true carnivores and prefer whole small prey including muscle, organs,
bones, feathers, and fur. They cannot survive on natural vegetarian diets. In Europe and
Australia ferrets are fed meat-based diets of whole prey (e.g. mice and rabbits) together
with raw meat (e.g. beef, chicken, and veal). This method of feeding is gradually becoming popular in the USA. However concern has been expressed over high carbohydrate levels in some processed ferret food. This is because an ideal ferret food should contain a minimum of 32% meat based protein, 18% fat and less than 3% fiber. Unlike minks; ferrets apparently have an aversion to the odor of fish. Therefore it is very important to avoid fish inclusion in the dissolution diet, because the primary end-point of this investigation was to determine the feasibility of dissolving ferret uroliths by modifying lithogenic and litholytic ingredients so as to produce a dietary prescription that would be safe, effective, and palatable. So if fish is included that might affect the determination of the effectiveness as well as the palatability of the dissolution diet since ferrets will not consume the diet.
CHAPTER 2

HISTORY OF STRUVITE UROLITHIASIS IN FERRETS
Chapter 2

HISTORY OF STRUVITE UROLITHIASIS IN FERRET

Magnesium ammonium phosphate hexahydrate (MAP) is commonly called struvite. The term struvite is an eponym coined in 1845 by Ulex, a Swedish geologist, in honor of H.C.G. von Struve (1772-1851), a Russian diplomat and naturalist. The name Struvite was given to the mineral because the crystal is composed of magnesium, ammonium and phosphate hexahydrate (MgNH4PO4·6H2O). Prior to that time, struvite was sometimes referred to as "guanite" because the mineral was detected in bat guano. Struvite is also sometimes referred to as “Triple phosphate” due to an antiquated erroneous belief that the phosphate ion was bonded to 3 positive ions instead of just magnesium and ammonium.

In cats and ferrets struvite uroliths are normally white, cream or light brown in color. They can occur singly or in multiples. Their surfaces may appear red due to hematuria or rarely green due to coloration by biliverdin which is a bile pigment. They are usually opaque, round, elliptical, or faceted; some may grow in the shape of renal pelves, ureters, bladder, or urethra. There are two types of struvite uroliths: sterile and infection-induced. In cats and ferrets, struvite uroliths are usually pure and occur predominantly in the lower urinary tract. On the other hand struvite uroliths in dog and human are often not pure.
While urinary tract infections with urease-producing microbes appear to be the most important cause of infection-induced urolithiasis in man and dogs, diets appear to be a major cause of sterile uroliths in cats and ferrets. The following microbes have been isolated from infection-induced struvite uroliths – Staphylococcus and proteus spp. There is speculation that ureaplasma may also be involved but because of the difficulty of growing it and because it cannot be gram stained this has not been established.

In cats the following risk factors may be associated with the sterile struvite struvite uroliths: gender, age, breed, reproductive status, hydration status, urine pH, and dietary composition, season of the year, anatomic location, and genetics. These risk factors may contribute to over-saturation of urine with magnesium, ammonium, and phosphoric acid.

Struvite uroliths may be suspected in cats and ferrets by a combination of history, clinical signs, urinalysis, urine culture, ultrasonography, and/or radiography. Knowledge of these predisposing factors that promote formation of uroliths is a key to development of safe and effective non-surgical treatment methods.

The advent of safe and effective diet therapy to induce dissolution of sterile struvite uroliths in cats, and the striking parallels between ferret and feline urolithiasis, prompts the question as to the safety and efficacy of diet-induced dissolution and prevention of sterile struvite urolith formation in ferrets. The primary goal of this study is to compare features of sterile struvite uroliths in cats with those in ferrets. Results of
these comparisons may provide a logical evidence-based rationale for clinical trials to
determine the safety and efficacy of diet-induced dissolution of sterile struvite uroliths in
ferrets.
CHAPTER 3

EPIDEMIOLOGY OF STRUVITE UROLITHS IN FERRETS
CHAPTER 3

EPIDEMIOLOGY OF STRUVITE UROLITHS IN FERRETS

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SUMMARY

**Objective**—To determine that the predominant mineral type in naturally occurring ferret uroliths was struvite and to determine if age, breed, gender, reproductive status, geographic location, season, and anatomic location were risk factors associated with urolith formation in ferrets. A primary goal was to compare features of struvite uroliths in cats with those in ferrets, and to determine if these results provide a logical evidence-based rationale for clinical trials of the safety and efficacy of diet-induced dissolution of struvite uroliths in ferrets.

**Design**—Retrospective case-control study

**Animals**—408 case ferrets from the Minnesota Urolith Center (MUC) and 6,528 control ferrets from the Veterinary Medical Data Base (VMDB) from 1981 through 2007.

**Procedure**—Historical information was obtained about each ferret. The association between these factors and outcome (struvite urolith formation) was statistically assessed.

**Results**—Sterile struvite was the predominant mineral in ferret uroliths. Neutered male ferrets had increased risk of developing sterile struvite uroliths. A significant association was also found between advancing age and the detection of struvite uroliths. Ferret struvite uroliths were more likely to be retrieved from the lower urinary tract than from the upper the urinary tract.

**Conclusion and Clinical Relevance**—Knowledge of predominant mineral type in uroliths along with insight into etiologic, demographic, and environmental risk and
protective factors for urolithiasis may facilitate development of surveillance strategies that result in earlier detection of uroliths in ferrets. Modification of risk factors including dietary risk factors may help to minimize urolith formation, dissolve existing uroliths and minimize urolith recurrence.
INTRODUCTION

Ferrets are becoming increasingly popular as household pets. As the population of pet ferrets increases, uroliths are being recognized with increased frequency. For example in 1981, the Minnesota Urolith Center analyzed uroliths from only 2 ferrets. In comparison, in 2007, uroliths from 176 ferrets were submitted for analysis. As the frequency of detection of uroliths in ferrets increases, knowledge of different mineral types affecting ferrets and associated risk factors for urolith formation are needed to develop effective diagnostic, management and prevention strategies. Because we have limited access to ferrets with uroliths, we have directed our efforts toward evaluation of the epidemiologic features of this disorder.

The purpose of this retrospective case-control study was to determine the predominant mineral type in naturally occurring ferret uroliths submitted to the MUC, and to determine if the ferret’s age, breed, gender, reproductive status and geographic location, season of detection, and location within the urinary tract were risk factors associated with struvite urolith formation in ferrets. Risk factors associated with sterile struvite uroliths were then compared with risk factors associated with sterile struvite uroliths in cats with the goal of determining if ferrets would be candidates for dietary trials for diet-induced sterile struvite urolith dissolution.
MATERIALS AND METHODS

Cases--Medical records of urolith submissions to the MUC were reviewed. Cases consisted of 408 ferrets with uroliths submitted by veterinarians in the USA to the MUC between January 1, 1981 and December 31, 2007. Uroliths retrieved from ferrets evaluated at MUC were counted only once for each year. To minimize confounding of the data by inclusion of ferrets with a history of recurrent uroliths, data related to recurrences were excluded.

Controls--Controls consisted of 6,528 ferrets without urinary tract disorders admitted to veterinary teaching hospitals in the United States between January 1, 1981 and December 31, 2007. They were identified by searching the records of the VMDB. Since preliminary evaluation indicated that uroliths were not detected in ferrets less than 2 months of age, ferrets less than 2 months were also excluded from the study.

Urolith analysis--The mineral composition of uroliths was determined by optical crystallography. When the composition of uroliths could not be determined by optical crystallography, the mineral composition was determined by infrared spectroscopy. Only ferrets with uroliths composed of at least 70% of the primary mineral were included. Uroliths containing nuclei and shell of different mineral types were classified as compound. Uroliths without a nidus or shell and containing < 70% of a single mineral component were classified as mixed.

Evaluation of struvite uroliths for microbes--Using a table of random numbers, struvite uroliths from 10 different ferrets were randomly selected from the collection of 272 archived air-dried struvite urolith submissions. They were immersed in separate
containers of 2% formalin for 12 hours to fix non-crystalline matrix components. These uroliths were then immersed in a decalcifying solution containing 1% dilute hydrochloric acid and 95% ethylene-diamine-tetra-acetic acid for 10 minutes. The demineralized uroliths were placed in one-piece tissue cassettes imbedded in paraffin, and stored at room temperature overnight. Then they were cut at 6-microns thickness with a microtome. A section of each struvite urolith was then stained with hematoxylin and eosin as well as gram stained. All sections were examined by light microscopy to detect bacteria. In similar fashion, 50 uroliths were randomly selected for bacterial aerobic culture using a technique previously described.

**Statistical Analyses**—Standard statistical software was used to determine descriptive statistics (mean and standard deviations) of age (≥ 2 months to < 6 months, 6 months to < 1 year, 1 year to < 2 years, 2 years to < 4 years, 4 years to < 7 years, and ≥ 7 years), gender, reproductive status, and geographic location of ferrets, anatomic location of the urolith within the urinary tract, and the season of urolith submission.

Crude odd ratios, adjusted odd ratios and logistic regression were calculated at 95% confidence intervals (CI) using Woolf’s method to assess whether, age, gender (male vs. female), reproductive status (neutered vs. intact), season (fall vs. spring vs. winter vs. summer), and geographical location (mid-west vs. southwest vs. west vs. south east vs. northeast) were associated with the occurrence of struvite uroliths. If any expected cell frequency in a contingency table was < 5, the Fisher exact test was used. In this study age group ≥ 2 months to < 6 months, females, intact reproductive status, winter, and
geographic area in the west of USA were arbitrarily chosen as reference groups for statistical analysis.

The 26-year study was arbitrarily grouped into 5 intervals (1981 to 1986, 1987 to 1992, 1993 to 1998, 1999 to 2004 and 2005 to 2007) to determine whether risk or protective factors changed over time. The Breslow-Day (B-D) statistic was computed to determine whether ORs were homogenous over the 5 time intervals.\textsuperscript{15} Odds ratios for age group, gender and reproductive status were calculated for each interval. The Mantel-Haenszel summary of OR was computed when Breslow-Day test was not significant. Values of $p < 0.05$ were considered significant.

Because of the absence of continuous variables, odds ratios and univariate logistic regression analyses were computed using a hierarchical well-formulated modeling method to find the best risk model for age group, gender, reproductive status, season, and geographic location. After adjustment for confounding factors and interactions were made, risk factors for developing uroliths were determined from the best model. Odd ratio estimates were considered to be significantly different from 1 if the 95\% CI did not encompass 1.0.\textsuperscript{16} On the basis of recommendations by Lilienfeld and Stolley\textsuperscript{17} we classified significant OR between 1.1 and 1.9, and ORs between 0.5 and 0.9 as weak associations. Likewise, we interpreted significant OR $> 2$ (i.e. risk) and $< 0.5$ (i.e. protective) as clinically (biologically) significant. All analyses were performed with standard software.\textsuperscript{D,e,f} Results were considered significant at values of $p < 0.05$ (95\% CI).
RESULTS

Urolith composition--Between 1981 and 2007, uroliths retrieved from 408 ferrets were analyzed. Two hundred and seventy-two (67%) were composed of struvite (Figure 1), 61 (15%) were cystine and 43 (11%) were calcium oxalate. The remaining, 32 (9%) were composed of ammonium urate (n=8), calcium carbonate (n=1), calcium hydrogen phosphate (n=3), magnesium hydrogen phosphate (n=1), non-crystalline materials (n=6), mixed minerals (n=4), silica (n=1), two or more minerals (compound n=4) and dried blood (n= 4). Uric acid, sodium urate, and xanthine uroliths were not observed. Of 272 uroliths classified as struvite, 239(88%) were composed of 100% struvite, 22 (8%) were composed of 90-99 % struvite, and 11 (4%) were composed of 70-89 % struvite.

Urolith histopathology and culture--Bacteria were not detected by aerobic culture (n=50) and Gram stained uroliths (n = 10). Likewise bacteria were not detected in hematoxylin and eosin stained urolith sections (n=10).

Age--Mean age ± SD of ferrets with struvite uroliths was 3.6 ± 2.8 years. The minimum age was 0.2 years and the maximum was 10.3 years. Struvite uroliths were found most commonly in the 2 to < 4 year (38%) age group followed by the 4 to < 7 year (34%) age group (Table 1; Figure 2). A significant association was found between advancing age and the detection of struvite uroliths (Table 1). From 1981 to 1986, the mean age was 2.5 ±1.8 years. From 1987 to 1992, the mean age was 3.5±0.7 years. From 1993 to 1998 the mean age was 3.5±1.6 years. From 1999 to 2004 the mean age was 4.9±1.6 years. From 2005 to 2007, the mean age was 5.5 ± 1.6 years.
Using 2 to 6 months as the baseline control group for comparison, 2 to 4 year old ferrets were 8.8 times (95% CI, 4.2-18.5, \( p < 0.0001 \)) as likely to develop struvite uroliths as ferrets in the control group. Ferrets 4 to < 7 years old were 6.6 times (95% CI, 3.1-13.9, \( p < 0.0001 \)) as likely to develop struvite uroliths compared to 2 to 6 month-old ferrets (Table 1; Figure 2).

For the Breslow-day test, it was necessary to divide ferrets into 2 age groups, those < 4 years old and those ≥ 4 years old. Ferrets ≥ 4 years old were 1.3 times as likely to develop struvite uroliths as were ferrets < 4 years old.

**Gender**—Of 260 ferrets with struvite uroliths for which gender was recorded, 73% (n=189) were males and 27% (n=71) were females. Male ferrets were 3.6 (95% CI, 2.5-5.1, \( p < 0.0001 \)) times as likely to develop struvite uroliths as were females (Table 1; Figure 3).

**Reproductive status**—Of 263 ferrets with struvite uroliths for which the reproductive status was recorded, 90% (n=237) were neutered and 10% (n=26) were reproductively intact. Neutered ferrets were 2.3 times (95% CI, 1.5-3.5; \( p < 0.0001 \)) as likely to develop struvite uroliths as were intact ferrets (Table 1; Figure 4).

**Anatomic location**—Of 258 struvite uroliths for which location was recorded, 198 (76%) were retrieved from the urinary bladder, 56 (20%) from the urethra, 1 (< 1%) from the kidney and, 3 (1%) from the ureters. Uroliths were voided by 14 (5%) ferrets. Ferret struvite uroliths were more likely to be retrieved from the lower urinary tract (bladder and

---

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urethra, n = 254) than from the upper the urinary tract (kidneys and ureters, n = 4) (Figure 5).

**Geographic location**--Of the 250 ferrets with struvite urolithiasis for which the geographical locations in the USA were recorded, 79 (31%) were from the mid-west, 54 (21%) were from the northeast, 70 (28%) were from the southeast, 24 (10%) were from the southwest, and 23 (9%) were from the west (Figure 5). Using the west as a reference point, a significantly greater number of ferrets with struvite uroliths resided in the Northeast (p < 0.0001; Table 1, Figure 6) at the time uroliths were retrieved.

**Seasonal distribution**--Of 272 ferrets with struvite uroliths for which the date was recorded at the time the uroliths were retrieved, 50 (18%) were recorded in winter, 60 (22%) were recorded in spring, 83 (31%) were recorded in summer and 79 (29%) were recorded in fall (Figure 7). Using winter (n = 60, 22%) as a reference season, probability values observed for summer (p = 0.43), spring (p = 0.98) and fall (p = 0.55) were not significant (Figure 7).

**Struvite urolith changes over time**--The number of struvite uroliths (N=272) submitted to the MUC increased from 3 (1%) observed between 1981 and 1992 to 47 (17%) observed between 2005 and 2007. The largest number (n=125) of struvite uroliths submitted to the MUC was observed between 1999 and 2004. During the same time interval, the mean age ± SD of ferrets with struvite uroliths increased from 2.5 ±1.8 between 1981 and 1992 to 5.5 ± 1.6 between 2005 and 2007.
**Odds ratios and logistic regression**--Age, gender, reproductive status and geographical location were adjusted as potentially confounding variables. Male reproductively neutered ferrets ($p < 0.0001$), ferrets 2 to < 4 years of age ($p < 0.0001$), ferrets 4 to < 7 years of age ($p < 0.0001$), and ferrets from the northeast ($p < 0.0001$) had increased risk for struvite urolithiasis (Table 1).

**Breed**--Unlike cats and dogs, ferrets are not classified according by breed. They are differentiated by their coat colors. Ferrets with different coat colors are often grouped together into one category designated as domestic ferrets. We were unable to classify ferrets by breed or coat color.
DISCUSSION

The results of this study support our hypothesis that struvite is the predominant mineral in ferret uroliths. Other investigators have reported that struvite was a common mineral in ferret uroliths, but to date we could not find any evidence-based studies identifying sterile struvite as the most common form of struvite in this species. In our study bacteria were not detected by light microscopic examination of sections of a representative subset of struvite uroliths stained with hematoxylin and eosin (n =10) and gram (n =10). Likewise, aerobic bacteria were not cultured from a different subset of struvite uroliths (n =50).

In our study, struvite uroliths were commonly retrieved from 2 to 7 year old ferrets. In a study of cats with sterile struvite uroliths reported in 2000, a similar range of ages (4 to 7 years) were found to be most common. Data derived from studies in dogs, cats, and humans suggest increased age is a risk factor for urolithiasis.

Results of our study of 272 ferrets with sterile struvite uroliths indicated that they were detected in male ferrets (73%) more often than in females (27%). These results may be related to the observations that the os penis of male ferrets is j-shaped and also that the distal urethra of male ferrets is smaller in diameter than the proximal urethra. These anatomic characteristics likely predisposed them to partial or total obstruction of the urethral lumen with uroliths. Struvite uroliths have been reported to be equally common in female and male cats.
A higher proportion of male and female neutered ferrets (79%) had sterile struvite uroliths compared to reproductively intact (21%) ferrets. However, there was no association between reproductive status and uroliths in that the same trend of association with neutering was observed in the control group.

Of the 258 sterile struvite uroliths, 98% were retrieved from bladder and urethra, while < 2% was retrieved from the kidneys and ureters. The occurrence of struvite uroliths from the lower urinary tract of domestic cats has been documented. Ferrets, dogs and cats are similar in that nephroliths are uncommon, while nephroliths are most common in man.

The results of our study supported the hypothesis that struvite uroliths may be influenced by region of submission because a significant \( p < 0.0001 \) number of ferret struvite uroliths were submitted to the MUC from the northeast region compared to the west. Our study was not designed to explore the reason(s) for this observation.

The results of our study did not support the hypothesis that different seasons of the year were associated with struvite submission. Since the population of ferrets involved in this study \( n = 272 \) was small, this observation should be verified by evaluating a larger population of clinical cases.

Of 272 uroliths classified as struvite, 239(88%) were composed of 100% struvite, 22 (8%) were composed of 90-99 % struvite, and 11 (4%) were composed of 70-89 % struvite. We interpret this data as evidence to support our conclusion that ferrets are similar to domestic cats in that struvite uroliths in both species are uncommonly
associated with urease producing microbial UTI. In contrast, struvite uroliths in dogs and humans typically form as a result of urinary tract infections with urease producing microbes. Infection-induced struvite found in dogs and humans typically contain 10 to 15% carbonate-apatite and 5 to 10% ammonium urate in addition to struvite. In our experience with tens of thousands of canine and feline struvite uroliths, infection-induced uroliths commonly contain carbonate-apatite and ammonium urate. In contrast, sterile struvite uroliths typically do not contain other biogenic minerals such as calcium carbonate-apatite and ammonium urate.

Struvite uroliths were classified as sterile struvite in this study on the basis of mineral composition, light microscopic appearance, and sterile cultures. In context of struvite urolithiasis, ferrets and domestic cats are remarkably similar. Both species are obligate carnivores. Both species form struvite uroliths that are sterile. In both species; infection-induced uroliths are uncommon. In both species, bacterial urinary tract infections also appear to be uncommon. The advent of safe and effective diet therapy to induce dissolution of sterile struvite uroliths in cats, and the striking parallels between ferret and feline struvite urolithiasis, prompts the question as to the safety and efficacy of diet-induced dissolution and prevention of sterile struvite urolith formation in ferrets. One of the primary goals of this study was to compare features of sterile struvite in cats with those in ferrets. In our opinion the results of these comparisons provide an evidence-based rationale for clinical
trials to determine the safety and efficacy of diet-induced dissolution of sterile struvite uroliths in ferrets.
Table 1--Crude and adjusted odds ratios (OR) and logistic regression for ferret struvite uroliths by age, gender, reproductive status, season and geographical location.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Descriptions</th>
<th>Crude OR</th>
<th>95% CI</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>≥ 6 months -&lt; 1yr vs. ≥2- &lt; 6months</td>
<td>1.4</td>
<td>0.6-3.2</td>
<td>0.7</td>
<td>0.2-2.2</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>1- &lt; 2yrs vs. 2-&lt; 6 months</td>
<td>3.4</td>
<td>1.7-6.8</td>
<td>2.9</td>
<td>1.3-6.8</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>2 - &lt; 5yrs vs. 2-&lt; 6months</td>
<td>7.6</td>
<td>4.0-14.3</td>
<td>8.8</td>
<td>4.2-18.5</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>4 - &lt; 7 yrs vs. 2-&lt; 6 months</td>
<td>6.9</td>
<td>3.8-13.1</td>
<td>6.7</td>
<td>3.1-13.9</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>≥ 7 yrs vs. 2-&lt; 6 months</td>
<td>4.5</td>
<td>1.9-10.6</td>
<td>4.4</td>
<td>1.6-12.1</td>
<td>0.004</td>
</tr>
<tr>
<td>Gender</td>
<td>Male vs. Female</td>
<td>3.5</td>
<td>2.5-4.9</td>
<td>3.6</td>
<td>2.5-5.1</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Reproductive status</td>
<td>Neutered vs. Intact</td>
<td>0.3</td>
<td>0.2-0.5</td>
<td>2.3</td>
<td>1.6-3.5</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Seasons</td>
<td>Fall vs. Winter</td>
<td>1.1</td>
<td>0.7-1.6</td>
<td>0.9</td>
<td>0.6-1.4</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>Spring vs. Winter</td>
<td>1.1</td>
<td>0.7-1.7</td>
<td>1.0</td>
<td>0.6-1.6</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Summer vs. Winter</td>
<td>1.4</td>
<td>0.9-2.0</td>
<td>1.2</td>
<td>0.8-1.8</td>
<td>0.44</td>
</tr>
<tr>
<td>Geographic location</td>
<td>Midwest vs. West</td>
<td>1.8</td>
<td>1.1-2.9</td>
<td>1.8</td>
<td>1.1-3.0</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Northeast vs. West</td>
<td>7.4</td>
<td>4.5-12.4</td>
<td>9.4</td>
<td>5.5-16.1</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Southeast vs. West</td>
<td>1.7</td>
<td>1.0-2.7</td>
<td>1.6</td>
<td>1.0-2.6</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Southwest vs. West</td>
<td>1.0</td>
<td>0.4-2.4</td>
<td>1.2</td>
<td>0.5-2.8</td>
<td>0.70</td>
</tr>
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</table>
Table 2-- Some comparisons of ferrets with domestic cats.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ferrets</th>
<th>Cats</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein requirement</td>
<td>Animal protein (30-40%)</td>
<td>Animal protein (15-30%)</td>
<td>1,30,31&amp;32</td>
</tr>
<tr>
<td>Urine pH range in health?</td>
<td>5.5 to 6.5</td>
<td>5.5 to 6.5</td>
<td>33</td>
</tr>
<tr>
<td>Range of urine specific gravity</td>
<td>1.001-1.089</td>
<td>1.001-1.084</td>
<td>33</td>
</tr>
<tr>
<td>Normal urine osmolality</td>
<td>Similar to cat.</td>
<td>≥ 3000mOsm/L</td>
<td>33</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>Uncommon</td>
<td>Uncommon</td>
<td>28,29 &amp;33</td>
</tr>
<tr>
<td>Penis</td>
<td>J-shaped os penis</td>
<td>Straight os penis</td>
<td>26,27 &amp;34</td>
</tr>
<tr>
<td>Struvite uroliths</td>
<td>Sterile struvite</td>
<td>Sterile struvite</td>
<td>25,28,29&amp;33</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>2 to 7 years</td>
<td>4 to 7 years</td>
<td>23</td>
</tr>
<tr>
<td>Neutered</td>
<td>(90%)</td>
<td>94% neutered</td>
<td>23</td>
</tr>
<tr>
<td>Struvite uroliths and gender</td>
<td>Males (73%); Females (27%)</td>
<td>Males (42%); Female (58%)</td>
<td>23</td>
</tr>
<tr>
<td>Anatomic locations of sterile struvite uroliths</td>
<td>Lower urinary tract (96%)</td>
<td>Lower urinary tract (95%)</td>
<td>23, 25&amp;29</td>
</tr>
<tr>
<td>Infection-induced uroliths</td>
<td>Uncommon (&lt; 5%)</td>
<td>Uncommon (&lt; 5%)</td>
<td>25,28,29 &amp; 34</td>
</tr>
</tbody>
</table>
Figure 1-- Typical struvite uroliths from the urinary bladder of neutered, 5-years old male ferret.
Figure 2--Frequency distribution of ferret struvite by age (Cases vs. controls).

Cases: n = 272
Controls: n = 6528

p = < 0.0001
Figure 3--Frequency distribution of ferret struvite uroliths by gender (Cases vs. controls; Gender for 12 cases were not recorded)
Figure 4--Frequency distribution of ferret struvite uroliths by reproductive status

(Cases vs. Controls; Reproductive status for 9 cases were not recorded).
Figure 5--Frequency distribution of ferret struvite uroliths by gender and anatomic location (Cases vs. controls; Anatomic locations of 14 cases were not recorded).
Figure 6-- Frequency distribution of ferret struvite uroliths by geographic location.

(Cases vs. controls; Geographic locations for 22 cases were not recorded).
Figure 7--Frequency distribution of ferret struvite uroliths by season.

(Cases vs. Controls)
CHAPTER 4

Cystine uroliths in ferrets: Perspective from the Minnesota Urolith Center.
CHAPTER 4

Cystine uroliths in ferrets: Perspective from the Minnesota Urolith Center.

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The main objectives of this retrospective study were 1) to evaluate the occurrence of cystine uroliths in ferrets, 2) to characterize the age, gender, and reproductive status of affected ferrets, and 3) to determine the anatomic location(s) of cystine uroliths. Records (requests for urolith analysis) of 435 ferrets submitted to the Minnesota Urolith Center were evaluated. Descriptive information was obtained about each ferret. The association between these factors and outcome (cystine uroliths formation) were assessed. Cystine uroliths comprised 16% (70 of 435) uroliths retrieved from ferrets and analyzed at Minnesota Uroliths Center from 1992-2009. Cystine uroliths were more common in male ferrets than females. A significant association was found between advancing age and the detection of cystine uroliths. Ferret cystine uroliths submitted to the Minnesota Urolith Center were retrieved from the lower urinary tract; none of the submitted uroliths were retrieved from the upper the urinary tract. Knowledge of the predominant mineral type in uroliths along with insight into etiologic, demographic, and environmental risk and protective factors for urolithiasis may facilitate development of surveillance strategies that result in earlier detection of uroliths in ferrets. Although a familial basis has been a documented risk factor for cystine urolithiasis in dogs and humans, genetic predispositions have not yet been proved in ferrets. The observation that cystine uroliths are associated with a familial cause in other species suggests that the parent stock of the ferrets in our study may have been inbred.
INTRODUCTION

Ferrets are becoming increasingly popular as household pets.\textsuperscript{8,9,10} as the population of pet ferrets increases, uroliths are being recognized with increased frequency. The number of uroliths submissions from ferrets to the Minnesota Urolith Center (MUC)\textsuperscript{a} has also progressively increased. The submission rates were 6\% (n=4) from 1992 to 1997, 24\% (n=17) from 1998 to 2003, and 70\% (n=47) from 2004 to 2009. As the frequency of detection of uroliths in ferrets increases, knowledge of the different mineral types affecting ferrets and associated risk factors for urolith formation are needed to develop effective diagnostic, management and prevention strategies.

The purpose of this retrospective study was to determine the frequency of occurrence of naturally occurring cystine uroliths in ferrets, and to determine if the ferret’s age, gender, and reproductive status were risk factors associated with cystine urolith formation. Anatomic location was also evaluated as a potential risk factors associated with cystine uroliths.
MATERIALS AND METHODS

Study population-Medical records of ferret urolith submissions to the MUC were reviewed. Between January 1, 1992 and December 31, 2009, uroliths from 435 ferrets were submitted to the MUC. Uroliths retrieved from ferrets evaluated at the MUC were counted once for each year.

Age, gender, reproductive status and location of uroliths within the urinary tract of ferrets were recorded. To facilitate comparison of these results with those from previous studies, ferrets with uroliths were divided into the following categories: 6 months to < 1 year, 1 year to < 2 years, 2 years to < 4 years, 4 years to < 7 years, and ≥ 7 years.

Anatomic location of uroliths was divided into 4 categories. Nephroliths and ureteroliths were classified as upper urinary tract; uroliths that were retrieved from the urinary bladder or urethra or that were voided were classified as lower urinary tract; uroliths retrieved from upper and lower urinary tract were classified as both. When the location of the uroliths was not provided, the location was classified as unknown.

Urolith analysis

The mineral composition of uroliths was determined by optical crystallography\textsuperscript{11} and when necessary by infrared spectroscopy.\textsuperscript{11} Uroliths containing at least 70% of a single mineral were classified as that mineral type. Uroliths containing nuclei and shells of different mineral types were classified as compound. Uroliths containing less than 70% of a single mineral component and without a nucleus or shell were classified as mixed.
Statistical analysis

Prevalence of mineral types of the uroliths was expressed as the frequency of ferret cystine uroliths submitted for analysis to MUC. Frequency also was used to describe the age, gender, and reproductive status, and anatomic location within the urinary tract.
RESULTS

**Urolith analysis**--Cystine uroliths comprised 70 (16%) of 435 uroliths retrieved from ferrets analyzed at the MUC from 1992-2009. Sterile struvite was the most common mineral type submitted between January 1, 1992 and December 31, 2009 (Table 1). Calcium oxalate was identified in (11%) of the uroliths. The remaining, 37 were composed of ammonium urate, calcium carbonate, calcium hydrogen phosphate, magnesium hydrogen phosphate, non-crystalline materials, mixed minerals, silica, two or more minerals (compound uroliths), purines, and dried blood (Table 1). Uric acid, sodium urate, and xanthine uroliths were not observed.

**Age**--The age of ferrets at the time cystine uroliths varied from 6 months to 7 years. The mean age ± SD of ferrets with cystine uroliths was 2.9 ± 1.5 years.

**Gender**--Male ferrets (77%; n=54) had more cystine uroliths than females (23%; n=16).

**Reproductive status**--Sixty-five (93%) of the ferrets with cystine uroliths were neutered, while 5 (7%) were reproductively intact.

Review of quantitative analysis of 70 cystine uroliths from ferrets submitted from 1992 to 2009 revealed that all were composed of 100% cystine (Table 1). Cystine uroliths were ovoid and smooth. They were light yellow to reddish brown in color and varied in size from 0.5 mm to several centimeters. All ferrets’ uroliths submitted to the MUC were obtained from the lower urinary tract: 56 (~80%) came from urethra, 11 (~15%) came from the bladder and 3 (~5%) of the uroliths were voided. The number of uroliths in each patient varied from one to more than 100.
DISCUSSION

Results of our study revealed that young male or neutered ferrets are at risk for cystin uroliths. However, without a control population it is not possible to infer that neutered ferrets were at higher risk than reproductively intact ferrets. Cystine is a relatively insoluble amino acid; and as a result, in high concentration it may precipitate and form stones. In dogs and humans, cystine is freely filtered by the glomeruli. The greater part of the filtered cystine is then actively reabsorbed by the proximal tubules. Cystinuria is characterized by impaired renal tubular reabsorption of cystine and other amino acids by the renal tubules. Cystine uroliths form in acid urine. Based on studies in human and dogs, it is likely that ferret cystinuria is an inborn error of metabolism.

Cystine was the second most predominant type of urolith in our series. The genetics, biological behavior, and therapy of cystine urolithiasis have been extensively documented in dogs and humans. Other investigators have reported that cystine rarely occurs in ferrets. We observed cystine in 0.1% (92 of 94,776) cat uroliths submitted to the MUC and 1% (3402 of 350,803) of dog uroliths submitted to the MUC between 1981 and 2007. In contrast, in this study cystine uroliths were observed in 16% (70 of 435) ferret uroliths submitted during a 17 year interval.

Apparent the biological behavior of cystine urolithiasis in ferrets has not been studied as we could only find empirical accounts of it mentioned in the English literature.

Although a familial basis has been well documented in dogs and humans, but genetic predispositions have not yet been proved in ferrets and cats. The observation that
cystine uroliths are associated with a familial cause in other species, and the relatively large percentage of ferret cystine uroliths compared to other species suggests that the parent stock of the ferrets in our study may have been inbred.
Table 1--Mineral composition of 408 ferret uroliths evaluated at the MUC from 1992 to 2009

<table>
<thead>
<tr>
<th>Mineral composition</th>
<th>Number of uroliths</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium ammonium phosphate 6H20</td>
<td>277</td>
<td>64</td>
</tr>
<tr>
<td>100%</td>
<td>244</td>
<td></td>
</tr>
<tr>
<td>70-99%</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Magnesium hydrogen phosphate 3H20</td>
<td>1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Cystine</td>
<td>70</td>
<td>16</td>
</tr>
<tr>
<td>Calcium oxalate</td>
<td>50</td>
<td>11</td>
</tr>
<tr>
<td>Calcium oxalate monohydrate</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70-99%</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Calcium oxalate dihydrate</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70-99%</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Calcium oxalate monohydrate &amp; dihydrate</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70-99%</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Calcium apatite</td>
<td>3</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Calcium hydrogen phosphate</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>100%</td>
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<td></td>
</tr>
<tr>
<td>70-99%</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>70-99%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonium urate</td>
<td>8</td>
<td>1.8</td>
</tr>
<tr>
<td>100%</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>70-99%</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Miscellaneous material</td>
<td>6</td>
<td>1.4</td>
</tr>
<tr>
<td>Mixed†</td>
<td>8</td>
<td>&lt;1.8</td>
</tr>
<tr>
<td>Compound ‡</td>
<td>6</td>
<td>&lt;1.4</td>
</tr>
<tr>
<td>Silica</td>
<td>1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Other</td>
<td>4</td>
<td>&lt;1</td>
</tr>
<tr>
<td>TOTAL</td>
<td>435</td>
<td>100</td>
</tr>
</tbody>
</table>

* Analyzed by polarizing light microscopy or infrared spectroscopy.
† Uroliths did not contain at least 70% of the mineral type listed; no nucleus or shell detected.
‡ Uroliths contained an identifiable nucleus and one or more surrounding layers of a different mineral type.
FOOT NOTES

a. Minnesota Urolith Center, University of Minnesota. 1352 Boyd Ave. St. Paul, MN 55108

b. Veterinary Medical Database, 1717 Philo Rd, Suite 15, Urbana, IL 61803-3007
   VMDB’s URL, http://www.vmdb.org “VMDB does not make any implicit or implied
   opinion on the subject of the paper or study”.

c. Decalcifying solution. Newcomer supply Co. 2505, Parview RD. Middleton, WI 53562

d. One-Piece tissue Cassettes. Surgipath Medical Ind., Inc. 5205 Route 12. PO
   Box 528 Richmond, IL 60071, USA.

e. Biostatistical Design and Analysis Center. Clinical and Translational Science
   Institute, University of Minnesota. 717 Delaware St. SE, Minneapolis, MN
   55414

f. EPI INFO, version 6.04b, Center for Disease Control, Atlanta, Ga. Available at


   1st, 2010.
ABBREVIATIONS

MUC   Minnesota Uroliths Center

CaOx  Calcium Oxalate

SD    Standard Deviation

OR    Odds Ratio

SAS   Statistical Analysis Software

CI    Confidence Interval

Ca_{10}(PO_4) CO_3 Calcium phosphate carbonate

Ca_{10} HPO_4 2H_2O Calcium hydrogen phosphate dihydrate

COM   Calcium oxalate monohydrate.

VMDB  Veterinary Medical Data Base
References


   In: Ettinger SJ, Feldman EC, eds. Textbook of veterinary internal medicine. 

8. Gehrke BC. Results of the AVMA survey of US pet-owning household on 

9. Boyce SW, Zingg BM, Lightfoot TL Behavior of Mustela putorius furo (the 

10. Jurek RM. A review of national and California population estimates of pet 
    ferrets. Calif. Dept. Fish and Game, Wildlife. Manage. Div., Bird and 
    Mammal Conservation Program Rep. 98-09; 11.
    Available at:www.dfg.ca.gov/wildlife/nongame/nuis_exo/ferret/ferret.html 

11. Ulrich KL, Bird KA, Koehler LA, et al. Urolith analysis: Submission, 
    393-400.


Appendix A – Figure 1- An adult ferret.
Appendix A– Figure 2 - Young ferrets.
Appendix B—Figure 1- Typical struvite uroliths from the urinary bladder of neutered, 5-years old male ferret.
Appendix C – Figure 1- Optical crystallography for urolith analysis.
Appendix C – Figure 2-Infrared spectroscopy for urolith analysis.
Appendix C - Table 1 Distribution of ferret struvite urolith by age (cases v. controls).

<table>
<thead>
<tr>
<th>Age category</th>
<th>Controls</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number/ %</td>
<td>Number/ %</td>
</tr>
<tr>
<td>2 to &lt; 6 months</td>
<td>1192 (19.4)</td>
<td>11 (4.5)</td>
</tr>
<tr>
<td>6 to &lt;1 year</td>
<td>977 (15.9)</td>
<td>13 (5.5)</td>
</tr>
<tr>
<td>1 to &lt;2 years</td>
<td>995 (16.2)</td>
<td>31 (13.1)</td>
</tr>
<tr>
<td>2 to &lt;4 years</td>
<td>1282 (20.8)</td>
<td>90 (38.1)</td>
</tr>
<tr>
<td>4 to &lt;7 years</td>
<td>1265 (20.5)</td>
<td>81 (34.3)</td>
</tr>
<tr>
<td>≥ 7 years</td>
<td>243 (4.0)</td>
<td>10 (4.2)</td>
</tr>
</tbody>
</table>
Appendix C – Figure 3(Left); Table 2 (Right) - Frequency of ferret struvite uroliths by geographic locations.

<table>
<thead>
<tr>
<th>Geographic locations</th>
<th>Cases %</th>
<th>Controls %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midwest</td>
<td>31</td>
<td>36</td>
</tr>
<tr>
<td>Northeast</td>
<td>21</td>
<td>6</td>
</tr>
<tr>
<td>Southeast</td>
<td>28</td>
<td>35</td>
</tr>
<tr>
<td>Southwest</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>West</td>
<td>9</td>
<td>17</td>
</tr>
</tbody>
</table>
Appendix C – Figure 4. Ferret struvite uroliths submission in USA (N=250)
Appendix C – Figure 5-FERRET UROLITHANALYSIS AT MUC 1981 to 2007

- Struvite, N=272 (67%)
- Cystine, N=61 (15%)
- Calcium Oxalate, N=43 (11%)
- Ammonium urate, N=8 (2%)
- Miscellaneous minerals, N=6 (2%)
- Mixed minerals, N=4 (<1%)
- Compound minerals, N=4 (<1%)
- Others (dried blood etc), N=4 (<1%)
- Calcium phosphate, N=3 (<1%)
- Calcium carbonate, N=1 (0.2%)
- Magnesium phosphate, N=1 (<1%)
- Silica, N=1 (<1%)

408 Ferret uroliths
N=408
Appendix C- Figure 6- Ferret urolith frequency distribution by gender & reproductive status.


272 ferret Struvite uroliths (67%)
- 239 (100%) males
- 33 (70-99%) females

61 ferret cystine uroliths (15%)
- 45 males (74%)
- 16 females (26%)

43 ferret CaOx uroliths
- 35 males (81%)
- 8 females (19%)

Other types of ferret uroliths
1. Calcium hydrogen phosphate (< 1%).
2. Calcium apatite (<1%).
3. Calcium carbonate (<1%).
4. Ammonium urate (2%)
5. Miscellaneous materials (2%).
6. Mixed (<1%).
7. Compound (<1%).
8. Silica (<1%).
9. Others (<1%).

260 ferrets with gender recorded.
- 189 males (73%)
- 71 females (27%)

55 intact ferrets (21%)
205 neutered ferrets (79%)
38 neutered ferrets (88%)
55 neutered ferrets (90%)
43 ferret Struvite uroliths.
61 ferrets
6 Intact ferrets (10%)
55 neutered ferrets (90%)
Appendix C- Figure 7-Ferret struvite urolith frequency distribution by gender (Cases v. controls).

6528 controls + 272 cases

- 3250 males (51%)
- 3167 females (49%)

6417 control ferrets

- 3794 neutered ferrets
- 2623 intact ferrets

- 189 males (73%)
- 71 females (27%)

260 cases ferret.

- 205 neutered
- 55 intact ferrets (21%)
Appendix C - Figure 8- Ferret struvite urolith after washing (Arrowed) and grinding
Appendix C- Figure 9- Negatively H& E stained struvite urolith (left) and a positive control (right; Arrowed).
Appendix C- Figure 10- Negative Gram stained struvite urolith (left) and the positive control (right; Arrowed).
Appendix C – Figure 11- Negative ferret struvite urolith culture (Left) and Positive control (Right; Arrowed)
Appendix C - Figure 12- Negative struvite urolith on blood agar (Left) and positive control on the right)
Appendix C- Figure 13-Negative struvite uroliths culture on MacConkey agar (Left) and positive control (right; Arrowed).
Appendix D- Table 1-Comparisons for the risks and protective factors for ferret’s sterile struvite urolith.

<table>
<thead>
<tr>
<th>Risk factors for MAP urolith in ferrets</th>
<th>Protective factors for MAP urolith in ferrets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Promote MAP urolith formation</td>
<td>Minimize MAP urolith formation</td>
</tr>
<tr>
<td>Age (2 to &lt; 7 years old) &amp; ≥ 4 years</td>
<td>Age (&lt; 2 and ≥ 7 years old) &amp; &lt; 4 years</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>Gender (female)</td>
</tr>
<tr>
<td>Reproductive status (neutered)</td>
<td>Reproductive status (intact)</td>
</tr>
<tr>
<td>Warm weather</td>
<td>Cold weather</td>
</tr>
<tr>
<td>Spring, fall and summer</td>
<td>Winter</td>
</tr>
<tr>
<td>Dehydration</td>
<td>Adequate water consumption</td>
</tr>
<tr>
<td>Dried food</td>
<td>Canned food</td>
</tr>
<tr>
<td>Dog food</td>
<td>Cat food</td>
</tr>
<tr>
<td>Plant sources of protein (i.e. corn)</td>
<td>Animal sources of protein</td>
</tr>
<tr>
<td>Concentrated urine</td>
<td>Less concentrated urine</td>
</tr>
<tr>
<td>Diet with excess magnesium &amp; phosphorus</td>
<td>Diet restricted in magnesium &amp; phosphorus.</td>
</tr>
<tr>
<td>CaOx control</td>
<td>Increase in CaOx urolith</td>
</tr>
<tr>
<td>Feeding excess protein</td>
<td>Inadequate protein</td>
</tr>
<tr>
<td>Urine stasis or withholding urine.</td>
<td>Regular urine voiding</td>
</tr>
<tr>
<td>Alkaline urine</td>
<td>Acidic urine</td>
</tr>
</tbody>
</table>
Appendix D-Figure 1- Typical cystine uroliths from the urinary bladder of neutered, 6-years old male ferret.