EPIDEMIOLOGICAL EVALUATION OF UROLITHS IN SELECTED ANIMALS

A DISSERTATION

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DEDICATION

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PRESENTATION OF THE DISSERTATION

My doctoral dissertation work consisted of three seemingly related topics in the general epidemiology of uroliths in selected animals: ferrets, goats, sheep and horses. These topics stimulated my interest because of the increasing use of these animals as household pets in the United States. Save ferrets all other animals involved in these studies are all herbivores. As the population of these animals increases as pets, uroliths associated with them are being detected with increased frequency. One explanation for this trend may be that pets may receive more intensive medical attention than more traditional livestock that are not kept as pets. Knowledge of the various mineral compositions of uroliths affecting ferrets, goats, sheep and horses and risk factors for urolith formation are needed to develop effective diagnostic, therapeutic, and preventive strategies. The dissertation presented here is therefore the study of the applications of epidemiological and biostatistical methods to determine the risk and protective factors associated with the increased use of these animals as house hold pets as well as the risks posed by herbs on urolith formation in them.

The studies in this dissertation are presented separately in ten main chapters. Chapter 1 deals with the review of literature. Chapters 2-3 delve on struvite and cystine uroliths in ferrets. Chapters 4-7 encompass calcium carbonate, silica, struvite uroliths, and global shifts in uroliths retrieved from goats and submitted to the Minnesota Urolith Center. Chapters 8-9 examine calcium carbonate and magnesium calcium phosphate carbonate uroliths in sheep. Chapter 10 explores calcium carbonate uroliths in horses. A single bibliography follows at the end of the chapters.

ABSTRACT

This dissertation examined the predominant minerals, as well as the risk and protective factors for urolith formation in ferrets, goats, sheep and horses. **S**terile struvite was the predominant mineral in uroliths in ferrets. The mean age of ferrets with struvite uroliths was 3.6 ± 2.8 years and range (0.2 to 9.8 years). Cystine comprised 70 of the 435 (16%) uroliths retrieved from ferrets. The mean age of ferrets with cystine uroliths was 4.1 ± 1.5 years and range (0.5 to 9 years). This was surprising, as one might expect an earlier onset of clinical manifestations of a probable genetic disorder, with the life span of ferrets being reported to be between 5 and 11 years. Cystine uroliths are not rare at all in ferrets as was originally thought. Genetic factors associated with cystine urolithiasis have not yet been reported in ferrets, but a familial pattern of inheritance determined to be a major underlying factor in cystine urolithiasis in dogs and humans suggests that this may be a factor in ferrets and that the parent stock of ferrets in the present study may have been inbred.

The mean age of goats with $CaCO_3$ urolith was 3.8 ± 1.8 years and range (0.3 to 14 years). Males are at increased odds for $CaCO_3$ urolith than females. Goats of African descent had a higher risk of developing calcium carbonate uroliths than did goats of non-African descent. The mean age of goats with silica uroliths was 3.1 ± 1.2 years and range (0.2 to 10 years). The bulk of goats with silica were fed grass/pasture (22%). Silica urolith submissions retrieved from goats and submitted to the MUC decreased from 20% between1984 and1998 to 11% between1999 and 2012. More than 57% percent of goats with struvite uroliths were < 1 year of age. Calcium carbonate uroliths rose significantly in North America but not in Europe and Asia from 40% in 1984 to 1998 to 45% in 1999

to 2012. Struvite and magnesium calcium phosphate apatite uroliths decreased significantly in North American continent respectively from 8% and 9% in 1984 to 1998 to 3% and 4% respectively in 1999 to 2012.

The mean age of sheep with $CaCO_3$ urolith was 3.4 ± 1.4 years and range (0.8 to 11 years). Calcium carbonate uroliths were common in Dorset, Suffolk and mixed breeds of sheep. These 3 breeds comprised 15 of 29 (52%) of case sheep with calcium carbonate uroliths with data available. The mean age of sheep with magnesium calcium phosphate carbonate (MCPC) uroliths was 1.6 ± 1.2 years and range (0.2 to 9 years). Rambouilette, Suffolk and Hamshire sheep breeds were at high frequency for magnesium calcium phosphate carbonate urolith formation. These 3 breeds comprised 23 of 31 (74%) of case sheep with MCPC uroliths with data available. Magnesium calcium phosphate carbonate uroliths were commonly retrieved from sheep < 1year of age and comprised 23 of 38 (60.5%) of sheep with this condition with age reported. MCPC is more common in male and sexually intact sheep.

The mean age of horse with $CaCO_3$ urolith during the study was 13 ± 1.8 years and range (0.3 to 36 years). Quarter and Thorough bred horses comprised 151 of 292 (52%) of all horses with $CaCO_3$ urolith. Horses between the ages of 6 and 17 years old comprised 197 of 323 (61%) horse cases with ages reported. Male horses had higher frequency for $CaCO_3$ uroliths compared to females. Neutered horses had higher frequency for $CaCO_3$ uroliths than sexually intact. Calcium carbonate uroliths in horses were more likely to be retrieved from the lower portions of the urinary tract (85%) of case horses than from the upper portions of the urinary tract (14%). Three (1%) were retrieved from both upper and lower tracts. A typical horse with $CaCO_3$ urolith is a neutered male between the ages of 6 and 17 years old. Seventy-six of 80 (95%) $CaCO_3$ uroliths were retrieved from horses between 1981 and 1997 and submitted to the MUC, while between 1998 and 2014 the amount retrieved from case horses were 227 of 278 (97%) CaCO3 and submitted to the same laboratory. This study identified risk factors associated with urolithiasis in ferrets, goats, sheep and horses; however, these associations do not allow conclusions regarding cause-and-effect relationships. Evaluation of risk and protective factors through epidemiological studies may be useful in the development of recommendations for the prevention and control of urolith formation in these selected animals.

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ABBREVIATIONS

MUC Minnesota Urolith Center

CI Confidence Interval

OR Odds Ratio

$CaCO_3$ Calcium carbonate

CPA Calcium phosphate apatite

CPC Calcium phosphate carbonate

CO Calcium oxalate

CC Calcium carbonate

COPD Compound

MCPC Magnesium calcium phosphate carbonate

MAP Magnesium ammonium phosphate

MAT Matrix

MCPA Magnesium calcium phosphate apatite

XAN Xanthine

COD Calcium oxalate dehydrate

COM Calcium oxalate monohydrate

GENERAL INTRODUCTION

Uroliths are macroscopic concretions that form within the urinary tract. They are primarily composed of minerals filtered by the kidneys, but can form from any substance that is sparingly soluble in urine. The disease caused by uroliths is called urolithiasis. For centuries, removal of uroliths from animals and man has been solely the duty of surgeons.

However, a paradigm shifts in management of canine uroliths which began to occur in 1973 has led to the improvement in our understanding of etiology and risk factors associated with urolithiasis in domestic animals. In 1978, a war was declared on urolithiasis. This war led to the development of the Minnesota Urolith Center in 1981, to investigate the causes, cures and prevention of urolithiasis. Using the state of the art science diagnostic techniques, the Minnesota Urolith Center has to date analyzed more than 1.3 million uroliths, which were submitted by veterinarians from more than 50 countries and from more than 100 species of companion animals, farm animals and wild animals. Over the past 43 years, great strides have been made in determining the mechanism of urolith formation as well as the roles risk and protective factor play in urolith formation in animals.

The Minnesota Urolith Center is unique in its ability to obtain data from large populations of animals, thus enhancing through epidemiological studies the discovery of demographic, environmental, and etiologic risk and protective factors associated this condition. Knowledge of predominant mineral type in uroliths in animals along with insight into etiologic, demographic, and environmental risk and protective factors for urolith formation may facilitate development of surveillance strategies that result in earlier detection of uroliths in animals. This study therefore, has attempted to further elucidate the risk and protective factors associated with urolith formation in these selected animals. Someday in future surgical removal of all types of uroliths in man and animals may become a subject of historical interest through knowledge obtained from epidemiological study such as this.

CHAPTER 1

A REVIEW OF THE ENGLISH LITERATURE

Incidence of uroliths in animals

Uroliths are macroscopic concretions that form within the urinary tract. They are primarily composed of minerals filtered by the kidneys, but can form from any substance that is sparingly soluble in urine. Urolithiasis is a common term used to designate the occurrence of uroiths in the urinary tract. This condition is responsible for considerable morbidity and mortality in the affected animals because it may result to life threatening urinary tract obstruction.[1-10] Reobstruction rates in animals with obstructive urolithiasis even after prompt and aggressive treatment may range from 29% to 30%. [4, 11] If obstructive urethroliths are not removed within 4-5 days, affected animal will die and the overall mortality rate can be up to 8.5%.[12] On the other hand, timely removal of urethroliths usually results in complete recovery.[12] Urolithiasis has been reported in dog[13], cat[14], ferret[15], goat[16], cattle[17], Llama[18], moose [8], sheep[19], horse [20], pig[21], rabbit[22], guinea pig[23] and man[14].

The incidence rate of urolithiasis reported in dogs seen at veterinary teaching hospitals in North America between 1980 and 1993 was 3, 628 of 676, 668 (0.53%). The proportion of dogs with urolithiasis admitted to veterinary hospitals in Germany at the same period was reported to be similar.[24] However, the incidence (annual rate of occurrence of new cases among the entire population at risk for the disease) of urolithiasis, mineral compositions and risk factors associated with this condition in ferrets, goats, sheep, and horses has not been fully established. Knowledge of the mineral composition and the underlying risk and protective factors for urolithiasis in domestic animals is a prerequisite for the formulation of consistently effective medical therapy to prevent the condition.

Minnesota Urolith Center (MUC) has been tracking the risk and protective factors as well as the occurrence of uroliths of domestic and exotic animals for more than 30 years. Since inception in 1984, MUC has quantitatively analyzed uroliths from over 1.3 million domestic and exotic animals. [8] On June 22, 2015, Minnesota Urolith Center (MUC) celebrated the analysis of the one millionth urinary stone (urolith) since the center embarked on reducing the worldwide incidence of urolithiasis in animals more than 30 years ago. This historic urolith came from a 4-year-old neutered male Shih Tzu, named Snickers, which was treated at Dogwood Animal Hospital in Fayetteville, in North Carolina. The most effective treatment and prevention protocols for urolithiasis are based on knowledge of the primary mineral type comprising the uroliths and associated risk and protective factors. All these are high on the priority list of the MUC.

Goats, ferrets, and horses as pets, and sheep as pet in the making

In addition to the traditional pets like dogs, cats and horses, there is a growing body of evidence to show that goats[25-27]and ferrets [28-30] are now increasingly used as pets in the United States. We predict that sheep will soon join the pet animals group in the USA. As these animals are increasingly used as pets, they are more likely to receive more veterinary care when sick, compared to animals kept for other purposes. They are also more likely to be kept in small quarters such as apartment complexes or town homes. As more and more ferrets, sheep, goats and horses are used as pets, more uroliths associated with them are seen more frequently by veterinarians. As the frequency of detection of uroliths in these animal's increase, knowledge of the different mineral types and associated risk and protective factors for urolith formation will be needed to develop effective diagnostic, management, and preventive strategies.

Formation of uroliths

The urinary system is designed to rid the body of waste products in soluble form. However, some waste products are sparingly soluble and sometimes precipitate out of solution to form crystals. Microscopic minerals which precipitate within urine are referred to as crystals. Crystals are like very fine sands and their presence in urine is known as crystalluria. The presence of crystalluria does not always leads to disease, although it does represent that the urine is supersaturated with mineral compound which is evidence that a risk exists for urolith formation. Urine crystal formation can occur in both in- vivo and in- vitro situations. Factors that affect in- vivo crystal formation are concentration of crystallogenic substances in urine and the volume of water in which they are suspended, urine pH, solubility, excretion of diagnostic agents, medications (i.e. sulfonamides), diet and rate of urine flow.

On the other hand, factors that influence in- vitro crystal formation includes temperature, evaporation, urine pH and technique of sample preparation (e.g., centrifugation compared to noncentrifugation and volume of urine examined) as well as method of preservation. In vitro changes which may occur in collected urine may enhance formation or dissolution of crystals. Although, these changes may enhance detection of some types of crystals (i.e., for instance, acidification to cause precipitation of cystine), in vitro crystal formation may have no clinical relevance to in- vivo formation of crystals in urine.

Urolith formation in animals conceptually involves two complimentary processes namely: crystal initiation and crystal growth.[24]In general most researchers have accepted the hypothesis that initiating events for uroliths formation are not the same for all mineral types. In addition, factors that initiate urolith formation in animals such as the magnitude of renal excretion of crystalloids, urinary pH and /or crystallization inhibitors or promoters which are present in urine may be different from those that inhibit their growth.

The first step in uroliths pathophysiology is formation of a crystal nidus (or crystal embryo) [24]This initiation phase of urolith formation is called nucleation. Nucleation is suggested to occur either spontaneously or on a pre-existing nidus. This process is dependent on supersaturation of urine with lithogenic crystalloids and consists of precipitation of small submicroscopic aggregates of ion groups in a process called nucleation. The inciting factor and the precise sequence of events that lead to the formation of most types of uroliths are still not fully understood. However, factors that may influence the degree of urine supersaturation include the magnitude of renal excretion of crystalloids, urinary pH and /or crystallization inhibitors or promoters which are present in urine.

The driving force that is involved in the process of nucleation is supersaturation, whereas the restraining force is the energy required for new surfaces to be formed. While nucleation creates a solid-liquid surface, surface tension tends to tear apart small groups of particles [24]Because the magnitude of the effect of surface tension is not directly proportional to the radius; larger groups of particles have less of a tendency to disperse back into solution unlike smaller groups of particles. Once a critical size nucleus is formed, its composite groups of particles remain in stable condition and continue to grow. The probability that groups of particles that are larger than critical size will form increases at greater degrees of supersaturation. In other words, when the driving force supersedes the restraining force for uroliths formation, nucleation tends to occur.

Solutions in the labile range of supersaturation readily generate critical size particles and thus permit spontaneous nucleation. Because nucleation occurs under this condition without involvement of pre-existing particles or surfaces, this process is called homogeneous nucleation .[24, 31-33] On the other hand heterogeneous nucleation occurs when crystal nuclei form on pre-existing surfaces. This phase can take place in metastable supersaturations. The new crystal nucleus only has to overcome part of the interfacial surface tension to form, so nucleation can occur at a lower degree of supersaturation. Since precipitation is more likely if heterogeneous nucleation occurs in urine, it may be very difficult to identify possible nucleation surfaces or niduses in the urine of urolith-formers. Foreign bodies such as suture materials and indwelling catheters are known to play very vital roles in nucleations. In the absence of such obvious initiating surfaces, however, niduses have been difficult to identify.[24] However, nucleation may occur on another crystal type in a process often times referred to as epitaxy.[25-26]Although, matrix has been implicated as the initiating surface upon which heterogeneous nucleation may occur, but the role of matrix in the whole process remains unclear .[24,31-33] Also necrotic debris and desquamated cells have been implicated to play some roles, but they have not been found at the central urolith nucleus as yet .[24,31-33]

Once nucleation is accomplished, crystal growth may continue provided the surrounding fluid remains supersaturated. Urolith particles may grow through the process of the addition of further ion groups onto the surface or by aggregation of crystals. It is believed that at low supersaturation, the addition of further atoms or ion groups is usually at spiral fault lines on the crystal surface. In this process less energy is required for deposition of crystals along fault line than commencement of a new layer. At high supersaturation, addition of further atoms or ion groups is more random and may occur anywhere on the surface of the crystal. Small particles tend to aggregate readily because in the aggregation process, the surface-to-mass ratio is reduced, causing energy to be released**.**

Because small particles in solution frequently collide, there is ample opportunity for aggregation to take place. Citrate, pyrophosphate, chondroitin sulfate, acidic peptides and ribonucleic acid have been observed to have inhibitory activity in calcium phosphate and calcium oxalate uroliths formation in humans[34-35] but the relative importance of each has not been clearly established. It is however believed that inhibitors act by occupying growth sites on the surface of crystals, thereby preventing the addition of further atoms or ion groups. Inhibitors exert this effect at low concentration. Inhibitors of crystal growth appear to be important in stone disease in humans because, when urine inhibitory activity and supersaturation are taken into consideration, there appear to be a clear-cut division between the two groups (both groups have individuals with relatively high and low supersaturation), but high saturation combined with low inhibitory activity in urine clearly identifies persons at risk for formation of uroliths. Similarly, low supersaturation with high inhibitory activity identifies people with a low risk of urolith formation. The balance between the two determines the likelihood of uroliths formation in man or domestic animal species. [24, 31-33]

As far as the transit time is concerned, urine supersaturation and cryastalluria are normal phenomena observed in dogs. As long as crystals continue to move at the same velocity as urine, urolithiasis does not occur. With reference to the process of physical chemistry, it is unlikely that a crystal particle could grow to a size large enough to delay its passage down the ureter unless some predisposing factors delayed its passage, allowing particles growth to continue. [36-39] As a result, nephroliths are most likely the result of fixed particles that initially grow in situ and then get detached into the renal pelvis.

Randall's plaques are thought to be accumulations of calcium crystals in subepithelial portions of the medullary collecting tubules and have been incriminated as a starting point for formation of calcium oxalate uroliths in human kidneys [36-39]The presence of glycosaminoglycan in the urine is thought to prevent crystals from adhering to the uroepithelium**.** When the uroepithelial surface is disrupted, crystal adherence to the uroepithlium will occur. It is probable that struvite nephrolith which occur secondary to urinary tract infection in dogs become established because ammonia produced by bacteria causes the alteration of the uroepithelial surface, thus promoting crystal adherence. On the other hand, urolith formation in the urinary bladder seems possible without invoking the need for a fixed-particle mechanism.   When pet that is well house-trained is left for a very long period between urination, they may be predisposed to prolonged crystal transit time and subsequently to poor emptying of the bladder. Both conditions are known risk factors for urocystolithiasis**.** Animals with an atonic bladder usually have a large residual bladder volume after micturition.[34]

As a result, larger crystals that may settle in the ventrum of the bladder may continue to grow if not voided and/or delayed voiding. If crystals are delayed in their transit route through the urinary tract, they may grow to such a size that they cannot readily pass through the ureters or urethra. Further growth of crystal nidus depends on: 1) whether or not it remains in the lumen of the excretory pathway of the urinary system, 2) whether the degree and duration of supersaturation of urine with crystalloids is identical or different from those in the nidus and 3) physical characteristics of the crystal nidus. [34]

Uroliths may pass through various sections of the excretory pathway of the urinary tract, they may be dissolved, become inactive or may continue to form and grow bigger. Clinical signs of urolithiasis may be the first indication of underlying systemic disorder, or defects in the structure or function of the urinary tract. When uroliths associated with clinical signs are allowed to remain untreated, they may result in serious sequelae.[34]

Epidemiological Studies

Epidemiological studies are important tools used in the identification of emerging trends in diseases. [40] In recent years, there has been a steady increase in the number of epidemiological studies in different veterinary publications describing the role that a variety of risk and protective factors play in the etiology and natural course of diseases such as urolithiasis. [41]A case in point is the epidemiological studies relating to the "epidemic" of canine and feline struvite and calcium oxalate urolithisis.[41]

Furthermore, the concept of risk factors associated with various human diseases has become a household word because of its popularity in newspapers and television news reports. Subsequently, clients in need of veterinary advice often times have questions about the significance of reported risk and protective factors as they relate to the type of urolithiasis affecting their animals.[40-42]Therefore a couple of questions will always be yearning for answers such as: (1) Risk and protective factors associated with urolithiasis, and how should we apply them in our efforts to diagnose, treat, and prevent urolithiasis? (2) Why are goats of African origin at increased risk for urolithiasis? (3) Why is sterile struvite the predominant urolith in ferret? (4) Why is cystinuria common in ferrets? (5) Why is a calcium carbonate uroliths predominant mineral in goats and horses? (6) Why are male ferrets, goats, sheep and horses at increased risk for urolithiasis? (7) Unlike in ferrets, goats and horses, why is the intact sheep more at risk for urolithiasis compared to neutered sheep? Etc These are some of the major questions that needed to be answered in the study reported here.

In terms of making good clinical decisions for diagnosis, treatment, and prevention of diseases, use of good evidence based medicine approaches for effective treatment and diagnosis decisions is very critical. [40-42] Evidence Based Veterinary Medicine (EBVM) in essence requires critical appraisal of scientific evidence along with clinical expertise and knowledge of cases involved. In line with this belief, the question such as how can one or more risk or protective factors associated with urolithiasis be identified? Most of the time it is very difficult to identify risk factors by classical experimental studies performed under controlled condition, because naturally occurring urolithiasis is affected by many different factors, some of which are known and some of which are unknown. For us to achieve this task, therefore we must design epidemiological studies to explore the relationship between these factors, of which the retrospective case-control and cross-sectional retrospective studies are some of the designs that are most frequently used.[40-42] Retrospective case-control studies consist of a defined sample of cases with the specific disease in question which is urolithiasis (i.e. calcium carbonate uroliths) and a defined sample of controls without the disease (urolithiasis) in question.

Advantage of these studies is that a number of potential risk or protective factors can be evaluated all at once. The frequency of past exposure of cases (urolithiasis) to suspected risk or protective factors is compared to the frequency of exposure of controls to a particular risk or protective factors. Retrospective case-control studies may explain why the disease (such as calcium carbonate urolithiasis) is more or less likely to occur in those cases with exposure to a particular risk or protective factors than the controls. Subsequently, results of case-control studies are more reliable than uncontrolled clinical studies. However, case-control studies do not prove cause and effect relationship. Confirmation of these relationships requires additional prospective or interventional studies to prove hypothesis derived from case-control studies (e.g. the hypothesis that urine acidifying diets alone do not cause calcium oxalate urolithiasis).[40-42]

Risk and protective factors

Risk and protective factors can be defined as the probability of disease developing in an individual during a specified time interval. Risk and protective factors can appear in different forms, but basically, they may be grouped into three categories: etiological risk factors (i.e. infectious agents, toxins and teratogens); demographic risk factors (i.e. species, breed, age, gender, and genetic predisposition); and environmental risk factors (i.e. living conditions, sources of water and food supply, and socio-economic status).

Each of these risk or protective factors may be involved in either a limited or significant role in the development or prevention of urolith formation. The probability of an animal developing a specific type of conditions such as urolithiasis when exposed to one or more risk or protective factors is often times expressed in terms of numerical odds or odds ratios or incidence (probability). Since not all risk and protective factors are created equally, the significance of risk or protective factors should not be assigned as an “all-or-none” or “always-or-never” interpretation. Most of the time, each risk factor contributes a limited role to the development of urolithiasis.[41]

Because individual risk factors may not be a factor in every exposed patient, identification of one event in a chain of etiological events is not the same as identifying the entire etiological chain of factors involved. Therefore, identification of the strength of the association is the key to narrowing down the possible risk or protective factors. As clinicians, we think of risk and protective factors as events that will affect the biological behavior of diseases such as urolithiasis.[41] Evaluation of risk and protective factors through epidemiological studies may be useful in the development of recommendations for the prevention and control of urolithiasis.[41]

Thesis Objectives

Most of the literature we reviewed on urolithiasis of ferrets, goats, sheep, and horses were mostly case reports and the few of them that were not case reports, were performed without controls.[3, 28] Instead, generalities they observed were based on uncontrolled empirical observations.

Minnesota Urolith Center has spent more than 30 years developing a state of the art facility with expertise in quantitative analysis of uroliths from all animals from around the world at no cost to the veterinarians. The ability of the MUC to be in possession of more than one million urolith samples in their data base obtained from veterinary communities from around the world provides a key resource for epidemiological study of this type. In addition, Minnesota Urolith Center offers latest information on urolith trends, treatment, and prevention strategies, thereby making the center a rallying point for veterinarians and clients alike. However, at this time essentially not much is known regarding the biological behavior and genetic link of urolithiasis in ferrets, goats, sheep, and horses. Also not much is known about diets and natural habitats of goats of African origin and ferrets. Lastly medical or dietary dissolution of uroliths is very advanced in dogs and cats but only in their rudimentary stages in ferrets, goats, sheep, and horses thereby making this study very important now than ever before.

This study is expected to fill the critical voids existing in our understanding of the biological behavior of urolithiasis in ferrets, goats, sheep, and horses. Identification of the risk and protective factors for uroliths formation in ferrets, goats, sheep, and horses will be the expected short term goal of this study. The long term goal of this study will be the evaluation of these risk and protective factors for the purpose of dietary prevention and dissolution of these uroliths in ferrets, goats, sheep, and horses. This study will also explore whether goats of African descent, ferrets, sheep and horses may be suitable models to investigate predispositions to urolithiasis. The information generated from this study will lead to complete reassessment of our prior and present views on risk and protective factors associated with urolithiasis in ferrets, goats, sheep, and horses.

CHAPTER 2

Epidemiology of struvite uroliths in ferrets: 272 cases

(1981-2007).

SUMMARY

The objectives of this study were to confirm that the predominant mineral in naturally occurring uroliths in ferrets was struvite; to determine whether breed, age, sex, reproductive status, geographic location of urolith-formers, season, and anatomic location were risk factors associated with urolith formation in ferrets; to compare features of struvite uroliths in cats with those in ferrets; and to determine whether there was a logical evidence-based rationale for clinical trials of the safety and efficacy of diet-induced dissolution of struvite uroliths in ferrets. This was a retrospective case-control study. It involved 408 ferret with uroliths of which 272 were struvite uroliths obtained from the Minnesota Urolith Center and 6,528 control ferrets obtained from the Veterinary Medical Database. The procedure involved obtaining historical information about each ferret on age, sex, reproductive status, geographical location, season of uroliths submission, anatomic location of the uroliths and type of diet used. The association between proposed risk factors and outcome (sterile struvite urolith formation) was analyzed and assessed. The results showed that **s**terile struvite was the predominant mineral in uroliths in ferrets. Male ferrets were at increased risk than females. Neutered ferrets had a significantly increased risk of developing sterile struvite uroliths than sexually intact. A significant association was also found between increasing age and the detection of struvite uroliths. Struvite uroliths in ferrets were more likely to be retrieved from the lower urinary tract than from the upper urinary tract. Bacteria were not detected by aerobic culture (n=50) nor by histologic examination of Gram-stained struvite uroliths (n=10). Likewise, bacteria were not detected in H&E-stained struvite urolith sections (n=10). Mean age ± SD of ferrets with struvite uroliths was 3.6 ± 2.8 years (range, 0.2 to 9.8 years; median,

4.5 years). Struvite uroliths in ferrets were found most commonly in the 2 to < 4 year (38%) age group. 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. The clinical relevance of this study is that 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. [28-30]As the population of pet ferrets' increases, uroliths are being recognized with increased frequency. For example, in 1981, the Minnesota Urolith Center (MUC) analyzed only uroliths from 2 ferrets. In comparison, in the year 2007, uroliths from 176 ferrets were submitted for analysis [a]. As the frequency of detection of uroliths in ferrets' increases, knowledge of different mineral types of uroliths affecting ferrets and associated risk factors for urolith formation is needed to develop effective diagnostic, management, and preventive strategies.

The purpose of the study reported here was to determine the predominant mineral type in naturally occurring uroliths in ferrets submitted to the MUC, and to determine whether the ferret's age, sex, 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 in ferrets were then compared to risk factors associated with sterile struvite uroliths in cats with the goal of determining whether ferrets would be candidates for dietary trials for diet-induced sterile struvite urolith dissolution. Because we had limited access to ferrets with uroliths, we have directed our efforts toward evaluation of the epidemiological features of this disorder.

MATERIALS AND METHODS

Cases- Medical records of urolith submissions to the MUC were reviewed[a]. These records were obtained from 408 ferrets with uroliths of which 272 were struvite uroliths submitted by veterinarian in the United States between January 1, 1981 and December 31, 2007.

Controls—Control group consisted of 6,528 ferrets without urinary tract disorders admitted to veterinary teaching hospitals during the same period as above[b]. They were identified by searching the records of the Veterinary Medical Data Base, which is responsible for compiling patients' encounter data from nearly all North American Veterinary Medical Colleges. Because preliminary evaluation indicated that uroliths were not detected in ferrets < 2 months of age, ferrets < 2 months of age were excluded from both cases and controls in the study.

Urolith analysis - The mineral composition of uroliths was determined by optical crystallography.[42] When the composition of uroliths could not be determined by optical crystallography, the mineral composition was determined by infrared spectroscopy.[42] Only ferrets with uroliths composed of at least 70% of the primary mineral were included. Uroliths containing nuclei and shell(s) of different mineral types were classified as compound. Uroliths without a nidus or with a shell and containing < 70% of a single mineral component were classified as mixed.

Optical crystallography was performed using polarized microscope. In this method trained technicians removed specific crystals from several areas within the sectioned urolith with the aid of a stereomicroscope. The crystals were immersed in oils of known refractive index, and were viewed through a polarizing microscope. Based on the refractive index and crystalline properties, determinations of mineral compositions were made. The polarizing microscopes used in the study included the Olympus BX50 and the Nikon Eclipse 50i polarizing microscopes. Infrared spectroscopy was used to detect the vibrational characteristics of chemical functional groups. Diffused reflectance infrared Fourier transforms spectroscopy (DRIFTS) and /or Attenuated total reflection (ATR) allowed for determination of composition with mineral sample preparation. The resulting spectrum from either technique was compared with a special library of known compounds to determine the composition of the sample. Nicolet iS10 Infrared Spectroscope from the Thermo-Scientific Corporation was used in this study.

There were situations where additional quantative techniques were used when necessary to identify mineral composition of some of the cases. In these scenarios utilization of x-ray crystallography and electron dispersive spectroscopy which were available through collaborating laboratories at the University of Minnesota and elsewhere as was necessary.

Evaluation of struvite uroliths for microbes –By using a table of random numbers, struvite uroliths from 10 ferrets were randomly selected from the collection of 272 archived air-dried struvite uroliths submissions. They were immersed in separate containers of 2% formalin for 12 hours to fix noncrystalline matrix components. These uroliths were then immersed in a decalcifying solution containing 1% dilute hydrochloric acid and 95% EDTA for 10 minutes [c]. The demineralized uroliths were placed in one-piece tissue cassettes, [d] imbedded in paraffin, and stored at room temperature of 20ºC overnight. Then, they were cut at a thickness of 6-µm with a microtome and a section of each struvite urolith was stained with H&E as well as Gram stain. All sections were examined via light microscopy to detect bacteria. In similar fashion, 50 uroliths were randomly selected for bacterial culture by use of a technique previously described.[43]

Statistical Analyses - Standard statistical software [44 e, f, g] was, used to determine descriptive statistics of age, sex, reproductive status, and geographic location of ferrets; anatomic location of the urolith within the urinary tract; and season of urolith submission. Ferrets were assigned to 1 of 6 age groups (2 months to < 1 year, 1 year to < 2 years, 2 years to < 4 years, 4 years to < 7 years, and ≥ 7 years).

Crude ORs, adjusted ORs, and logistic regression were calculated at 95% CIs by use of the Woolf's method [45] to assess whether, age, sex (male vs. female), reproductive status (neutered vs. sexually intact), season[h] (fall vs. spring vs. winter vs. summer), anatomic location (lower vs upper urinary tract), and geographical location, [l] Mid-west vs. Southwest vs. West vs. Southeast 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.[46] In this study, age group 2 months to < 6 months, females, sexually intact reproductive status, winter, and struvite uroliths anatomic location in the upper urinary tract, and geographic area in the West of the United States 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 statistic was computed to determine whether ORs were homogenous over the 5 time intervals.[47]Odds ratios for age group, sex, and reproductive status were calculated for each interval. The Mantel-

Haenszel summary of OR was computed when results of the Breslow-Day test were not significant. Values of $P < 0.05$ were considered significant.

Because of the absence of continuous variables, ORs and univariate logistic regression analyses were computed using a hierarchical well-formulated modeling method to find the best risk model for age group, sex, reproductive status, season, and anatomic location, and geographic location. After adjustment for confounding factors and interactions was 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.[48] On the basis of recommendations by Lilienfeld and Stolley [49] 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 ORs >2 (i.e. risk) and < 0.5 (i.e. protective) as clinically (biologically) important. All analyses were performed with standard software.[44, e] Results were considered significant at values of $P < 0.05$.

RESULTS

Urolith composition—Between 1981 and 2007, uroliths retrieved from 408 ferrets were analyzed at the MUC of which 272 (67%) were composed of struvite, 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), ≥ 2 minerals (compound n=4) and dried blood (n= 4) (Table 1). 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) nor by histologic examination of Gram-stained urolith (10). Likewise, bacteria were not detected in H&E-stained urolith section (n=10).

Age—Mean ± SD age of ferrets with struvite uroliths was 3.6 ± 2.8 years (range, 0.2 to 9.8 years; median, 4.5 years). For all 408 ferrets, age ranged from 0.2 to 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 (**Figure 1; Table 1**). A significant (P < 0.001) association was found between advancing age and the detection of struvite uroliths. 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- month-old ferrets as the baseline control group for comparison, 2- to 4- year old ferrets were 8.8 times (95% CI, 4.2 to 18.5, P < 0.001) as likely to develop struvite uroliths as were ferrets in the control group. Ferrets 4 to < 7 years old were 6.7 times (95% CI, 3.1-13.9, P < 0.0001) as likely to develop struvite uroliths, compared to 2-to 6-month-old ferrets (Figure 1; Table 1).

For the Breslow-Day, it is 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.

Sex—Of 260 ferrets with struvite uroliths for which sex was recorded, 73% (n=189) were males and 27% (n=71) were females. Male ferrets were 3.6 times (95% CI, 2.5 to 5.1, P < 0.001) as likely to develop struvite uroliths as were females (**Figure 2; Table 1**).

Reproductive status—Of 263 ferrets with struvite uroliths for which the reproductive status was recorded, 237 (90%) were neutered, and 26 (10%) were reproductively intact. Neutered ferrets were 2.3 times (95% CI, 1.5 to 3.5, P < 0.001) as likely to develop struvite uroliths as were sexually intact ferrets (**Figure 3; Table 1**).

Anatomic location—Of 258 struvite uroliths for which location was recorded, 198 (76%) were retrieved from the urinary bladder, 56 (22%) from the urethra, 1 (< 1%) from the kidney and, 3 (1%) from the ureters. Uroliths were voided by 14 (5%) ferrets. Struvite uroliths in ferrets were significantly (P < 0.001) more likely to be retrieved from the lower urinary tract (bladder and urethra, n = 254) than from the upper the urinary tract (kidneys and ureters, n = 4) in both males and females (**Figure 4; Table 1**).

Geographic location—Of the 250 ferrets with struvite urolithiasis for which the geographical locations in the United States were recorded, 79 (32%) were from the Midwest, 54 (22%) 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; Table 1**). Using the West as a reference point, a significantly (P < 0.001) greater number of ferrets with struvite uroliths resided in the Northeast (P < 0.001; Table 3) 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 6**). Using winter (n=60, [22%]) as a reference season, probabilities observed for summer (p=0.43), spring (P= 0.98) and fall (P= 0.55) were not significant.

Struvite urolith changes over time—The number of struvite uroliths (n=272) submitted to the MUC increased from 3 (1%) between 1981 and 1992 to 47 (17%) 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 ± SD age of ferrets with struvite uroliths increased from 2.5 ±1.8 between 1981 and 1992 to 5.5 ± 1.6 between 2005 and 2007(median, 4 years; range, 0.2 to 9.8 years).

ORs and logistic regression—Age, sex, reproductive status and geographical location were adjusted as potentially confounding variables. Neutered ferrets (P < 0.001), ferrets 2 to < 4 years of age (P < 0.001), ferrets 4 to < 7 years of age (P < 0.001), and ferrets from the Northeast (P < 0.001) had increased risk for struvite urolithiasis (**Table 1**).

Breed—Unlike cats and dogs, ferrets are not classified according to breed. They are differentiated by their coat colors. [50] Ferrets with different coat colors are often grouped together into 1 category designated as domestic ferrets. We were unable to classify ferrets by breed or coat color.

Struvite urolith composition—Quantitative analysis of 272 struvite uroliths revealed that 239 (88%) were composed of 100% struvite, 22 (8%) were composed of 90% to 99% struvite, and 11 (4%) were composed of 70% to 89% struvite. The uroliths retrieved from ferrets were pyramidal and smooth, yellow or brown-white, and ranged from 0.5mm to several centimeters in diameter (largest uroliths from animals). The number of uroliths in each ferret varied from 1 to > 100.

DISCUSSION

The results of the present study support our hypothesis that struvite is the predominant mineral found in uroliths in ferret. Other investigators have reported [51-54] that struvite is a common mineral in ferret uroliths, but we could not find any evidence-based studies identifying sterile struvite as the most common form of struvite in this species. In the present study, bacteria were not detected via light microscopic examination of sections of a representative subset of struvite uroliths stained with H&E (n=10). Likewise, aerobic bacteria were not cultured from a different subset of struvite uroliths (n=50).

In the present study, struvite uroliths were commonly retrieved from 2-to 7-year-old ferrets. In a study of cats [55] 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[56, 57] in dogs, cats and humans suggested increased age is a risk factor for urolithiasis.

Results of the present 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 os penis of male ferrets is j-shaped, and also the distal portion of the urethra of male ferrets is smaller in diameter than the proximal portion of the urethra. These anatomic characteristics likely predisposed them to partial or total obstruction of the urethral lumen with uroliths. [58-59] Struvite uroliths have been reported to be equally common in female and male cats. [55]

A higher proportion of neutered male and female ferrets (79%) had sterile struvite uroliths, compared with the proportion for sexually intact ferrets (21%). However, there was no association between reproductive status and urolith formation 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 the bladder and urethra, and < 2% were retrieved from the kidneys and ureters. The occurrence of struvite uroliths from the lower urinary tract of domestic cats has been documented.[60-61] Ferrets, dogs, and cats are similar in that nephroliths are uncommon, whereas nephroliths are most common in humans. [56]

The results of the present study support the hypothesis that struvite uroliths may be influenced by region of submission because a significant ($P < 0.001$) number of ferret struvite uroliths were submitted to the MUC from the Northeast region, compared to the West. The present study was not designed to explore the reasons for this observation. Our study did not support the hypothesis that seasons of the year were associated with stuvite submission. Because the population of ferrets with struvite uroliths involved in this study (n=272) was small, this observation should be verified by evaluating a larger population of clinically affected animals.

Of the 272 uroliths classified as struvite in the present study, 239 (88%) were composed of 100% struvite, 22 (8%) were composed of 90% to 99% struvite, and 11 (4%) were composed of 70% to 89% struvite. We interpret these 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 urinary tract infections.[56] In contrast, struvite uroliths in dogs and humans typically form as a result of urinary tract infections with urease-producing microbes. [53] Infection-induced struvite uroliths found in dogs and humans typically contain 10% to 15% carbonate-apatite and 5% to 10% ammonium urate in addition to struvite.[56] 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. We studied the epidemiological features of ferrets with sterile struvite uroliths and compared them with the epidemiological features of sterile struvite uroliths retrieved from cats. There are many similarities. For example, both species are obligate carnivores.[62-65]Both species form struvite uroliths that are sterile. [19, 22-23] In both species, infection-induced uroliths are uncommon.[57,60-61] In both species, bacterial urinary tract infections also appear to be uncommon.[62,66] In healthy ferrets and cats, the urine pH range is similar (pH, 5.5 to 7).[66] Likewise, the urine specific gravity values in both are typical of carnivores (1.001 to

1.089).[66] In both species, urine osmolality is similar (≥ 3,000) mOsm/L.[66] Also in both species, neutering (≥ 90%)and lower urinary tract anatomic location of struvite uroliths (≥ 95%) are common.[55,60-61] Whereas the os penis in ferrets is j-shaped, the os penis is straight in cats. Both species have narrow lumen in the distal portion of the urethra.[58-59, 67]

The advent of safe and effective diet treatment to induce dissolution of sterile struvite uroliths in cats[30] and the parallels between struvite urolithiasis in ferrets and cats[68] prompts questions as to the safety and efficacy of diet-induced dissolution and prevention of sterile struvite urolith formation in ferrets. One of the goals of the present study was to compare features of sterile struvite in ferrets with those in cats. In our opinion, the results of those 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.

Biases and limitations: (1) Since our data was obtained from ferret's urolith submissions to the MUC, it is not likely to be a complete representative of all urolith submissions of all breed, age, sex, season and geographic location of all ferret populations.

(2) The urethras of male ferrets are much longer and narrower than females. Also the males have j-shaped penis which are absent in females. All these features may have contributed to more clinical manifestations of urethral obstruction in males compared to females and may have therefore influenced their selection as cases.

(3) Since the MUC uroliths analyses are performed at no cost to veterinarians this may have influenced the rate of ferret urolith submissions to the center.

(4) Because the sample size in this study was not very large we are unable to apply the conclusions obtained from our findings to the whole ferrets' population.

(5) Uroliths obtained from male ferrets in this study were retrieved from the lower portion of the urinary tract. Clinical signs are more likely to be observed when uroliths are located in the lower urinary tract than from the upper urinary tract. This may have constituted a form of bias in this study leading to higher number of lower urinary tract uroliths observed in this study compared to the upper portion of the urinary tract uroliths.

(6) This study may have included more clinical cases, and may not have included subclinical cases or cases that may have been euthanized due to urinary tract obstructions; as a result, this may have limited the use of results from this study to fully represent the population of all ferrets with uroliths because of this form of bias.

(7) Higher prevalence of uroliths may have been observed in pet ferrets because of the increase in the trend of keeping them as pets. As pets, they may have received more veterinary care when sick compared to ferrets kept for other purposes. This trend may have influenced the submission of uroliths from this group of ferrets used as pets compared to ferrets used for other purposes other than pet.

Table 1—Crude and adjusted ORs and logistic regression analysis of struvite uroliths in ferrets submitted to the Minnesota Uroliths Center [a] between1981-2007 by age, sex, reproductive status, anatomic location, season and geographic location (affected ferrets' vs control ferrets from the Veterinary Medical Database [b]).

Variables	Descriptions	Crude OR	95% CI	Adjusted OR	95% CI	P value
Age	≥ 6 months -< 1yr vs. ≥2- < 6months	1.4	0.6-3.2	0.7	0.2-2.2	0.57
	1 – < 2yrs vs. 2- < 6 months	3.4	1.7-6.8	2.9	1.3-6.8	0.011
	2 - < 4yrs vs. 2- < 6months	7.6	4.0-14.3	8.8	4.2-18.5	<.001
	4 - < 7 yrs vs. 2- < 6 months	6.9	3.8-13.1	6.7	3.1-13.9	<.001
	≥ 7 yrs vs. 2- < 6 months	4.5	1.9-10.6	4.4	1.6-12.1	0.004
Sex	Male vs. Female	3.5	2.5-4.9	3.6	2.5-5.1	<.001
Reproductive status	Neutered vs. Intact	0.3	0.2-0.5	2.3	1.6-3.5	<.001
Anatomic location	Lower tract vs. Upper tract	7.3	4.5-12.6	9.5	5.6-17.8	<.001
Seasons	Fall vs. Winter	1.1	0.7-1.6	0.9	0.6-1.4	0.55
	Spring vs. Winter	1.1	0.7-1.7	1.0	0.6-1.6	0.98
	Summer vs. Winter	1.4	0.9-2.0	1.2	0.8-1.8	0.44
Geographic location	Midwest vs. West	1.8	1.1-2.9	1.8	1.1-3.0	0.01
	Northeast vs. West	7.4	4.5-12.4	9.4	5.5-16.1	<.001
	Southeast vs. West	1.7	1.0-2.7	1.6	1.0-2.6	0.07
	Southwest vs. West	1.0	0.4-2.4	1.2	0.5-2.8	0.70

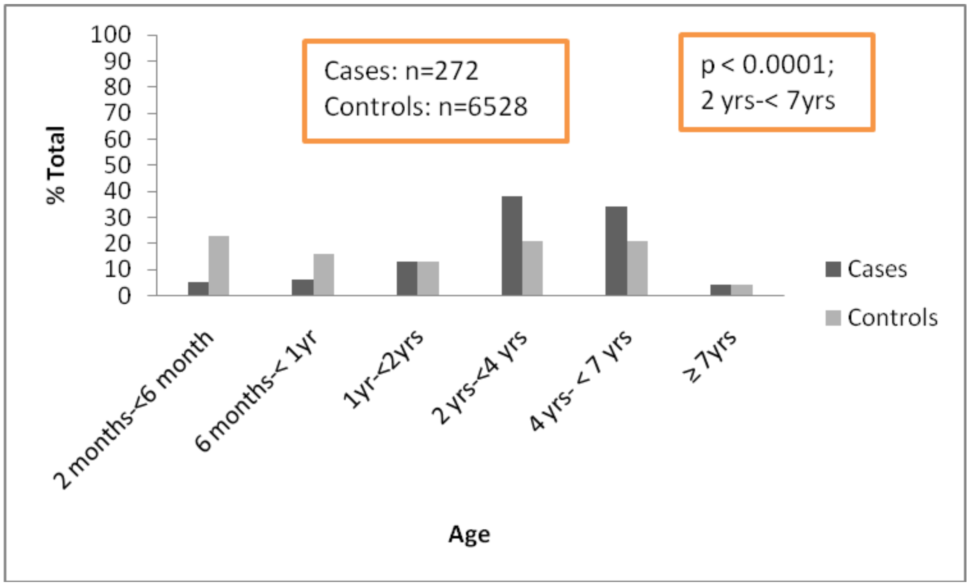

Figure 1—Age distribution for ferrets for which struvite uroliths were submitted to the Minnesota Uroliths Center [a] between 1981 and 2007 (cases) and control ferrets from the Veterinary Medical Database [b]).

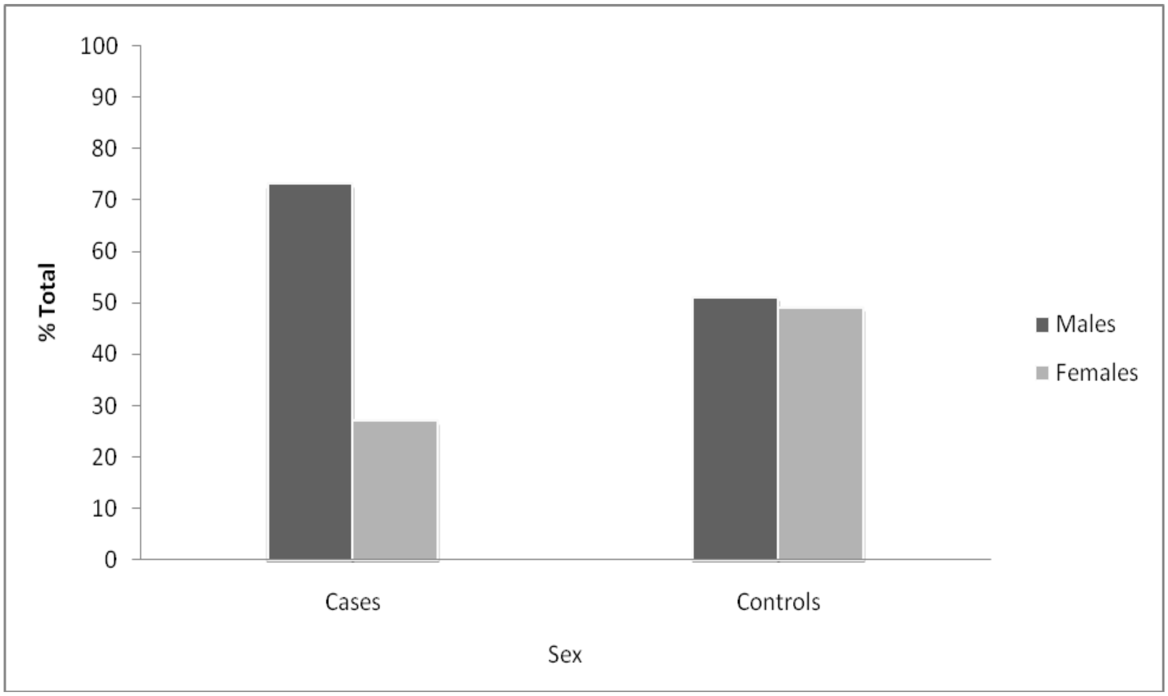

Figure 2—Sex distribution for ferrets for which struvite uroliths were submitted to the Minnesota Uroliths Center [a] between 1981-2007 (cases) and control ferrets from the Veterinary Medical Database [b]; sex for 12 cases was not recorded.

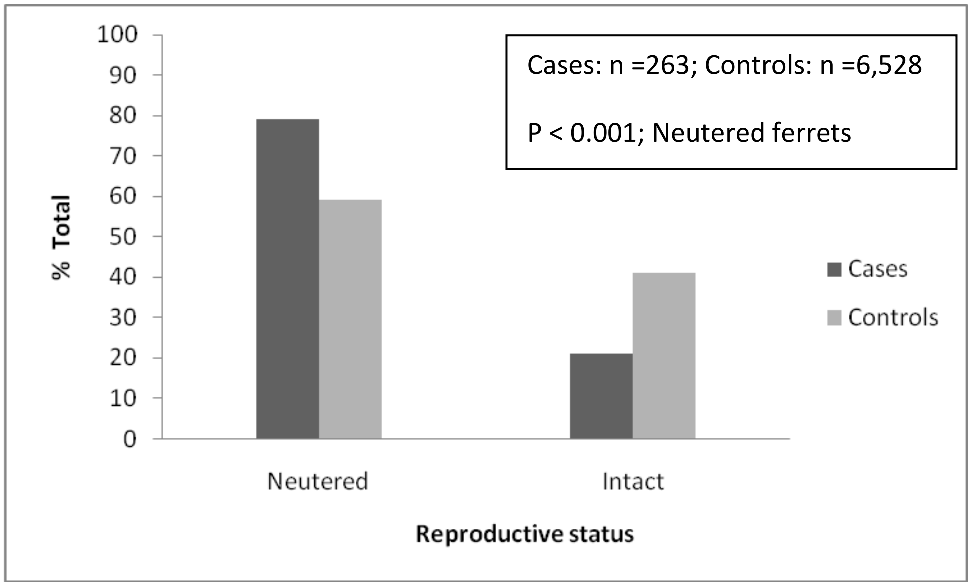

Figure 3—Reproductive status for ferrets for which struvite uroliths were submitted to the Minnesota Uroliths Center [a] between 1981-2007 (cases) and for by control ferrets from the Veterinary Medical Database [b] reproductive status for 9 cases was not recorded)

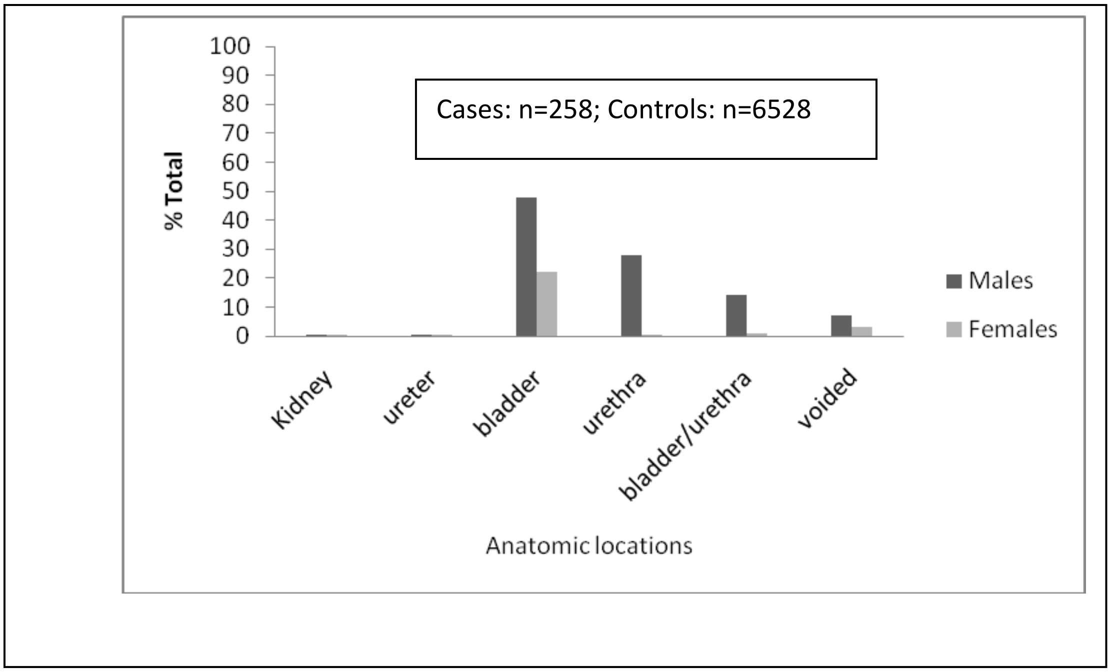

Figure 4—Frequency distribution by sex and anatomic location for struvite uroliths in ferrets submitted to the Minnesota uroliths Center[a] between 1981 and 2007; anatomic locations for 14 cases were not recorded).

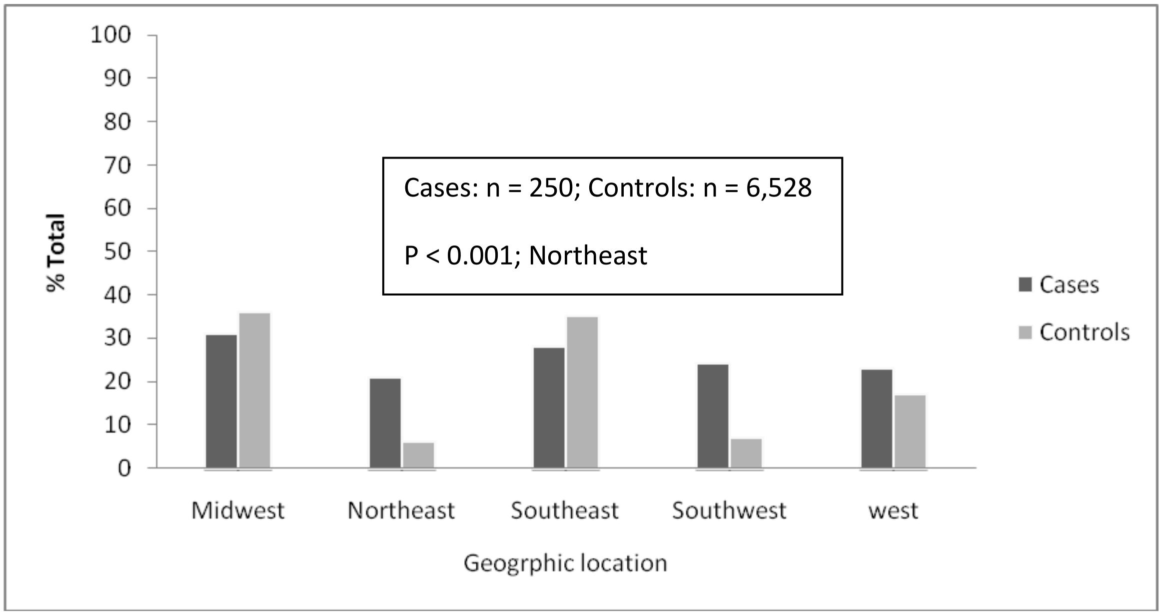

Figure 5—Geographic location for ferrets for which struvite uroliths were submitted to the Minnesota uroliths Center [a] between 1981-2007 (cases) and controls ferrets from the Veterinary Medical Database [b], geographic locations for 22 cases were not recorded).

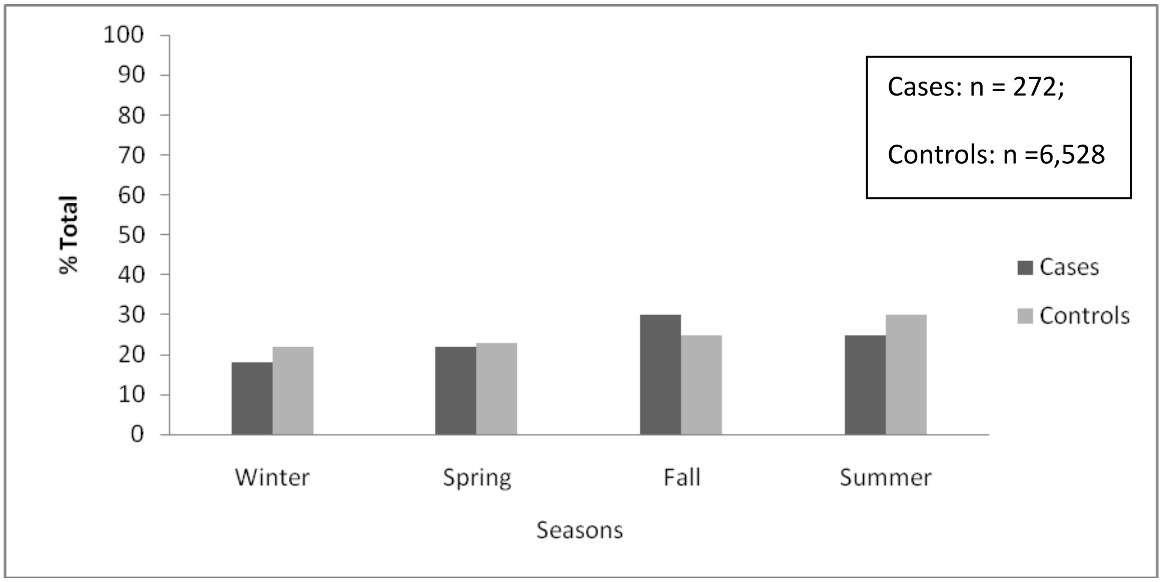

Figure 6—Season of examination for ferrets for which struvite uroliths were submitted to the Minnesota Uroliths Center [a] between 1981-2007 (cases) and control ferrets from the Veterinary Medical Database.[b]

Figure 7—Photograph of a tyical struvite uroliths from the urinary bladder of neutered 4-year-old male ferret (Mustela putorius furo). Scale indicates centimeters.

CHAPTER 3

Epidemiological evaluation of cystine urolithiasis in domestic ferrets (Mustela putorius furo): 272 cases (1992-2009)

SUMMARY

The objective of this study was to determine the prevalence of cystine uroliths in domestic ferrets with urolithiasis and determine whether breed, age, sex, reproductive status, anatomic location, and season of uroliths submission were risk factors associated with cystine urolith formation. The design was a retrospective cross-section case-control study. It involved 435 ferrets (Mustela putorius furo) with uroliths submitted for analyses to the MUC between 1992 and 2009, of which 70 were cystine uroliths.

Specific descriptive information about each ferret (age, sex, reproductive status, geographic location of ferrets, season of uroliths submission, anatomic location and type of diet used) were collected to determine whether specific risk factors were associated with the development of cystine uroliths. The mean age of ferrets with cystine uroliths was 4.1 ± 1.5 years and range (0.5 to 9 years). Cystine uroliths comprised 70 of the 435 (16%) uroliths from ferrets. Cystine uroliths were more common in male (n=54) than in female (16) ferrets. All cystine uroliths were retrieved from the lower portions of the urinary tract (bladder and urethra [n=67]) or were voided (3); none of the uroliths were retrieved from the upper portions of the urinary tract (kidney and ureters). Awareness of the prevalence of cystine uroliths along with knowledge of etiologic, demographic, and environmental risk and protective factors for urolithiasis may facilitate development of surveillance strategies that result in earlier detection of cystinuria. Genetic factors associated with this disease have not yet been reported in ferrets, but a familial pattern of inheritance determine to be a major underlying factor in cystine urolithiasis in dogs and

humans suggests that this may be a factor in ferrets and that the parent stock of ferrets in the present study may have been inbred.

INTRODUCTION

Ferrets (*Mustela putorius furo*) are becoming increasingly popular as household pets.[28-30] As the population of pet ferrets' increases, uroliths are being recognized with increased frequency. The number of ferret cystine urolith submissions to the MUC progressively increased from 4 in the period 1992 to 1997, to 19 in the period 1998 to 2003, and to 47 in the period 2004 to 2009.

As the frequency of detection of ferrets' uroliths increases, knowledge of their mineral types and associated risk factors for formation is needed to develop effective diagnostic, management, and prevention strategies. The purpose of the study reported here was to determine the prevalence of naturally occurring cystine uroliths retrieved from ferrets and submitted to the MUC and determine whether breed, age, sex, reproductive status, anatomic location within the urinary tract, and season of detection were risk factors associated with cystine urolith formation.

MATERIALS AND METHODS

Cases—Medical records of ferret uroliths submitted to the MUC[a] between January 1, 1992, and December 31, 2009, were retrieved for review. During that period, 435 ferret uroliths, of which 70 were cystine uroliths, were received from veterinarians in the United States. Because preliminary evaluation indicated that cystine uroliths were not detected in ferrets < 6 months of age, ferrets of this age were excluded from the study.

Controls—Controls consisted of 6,426 ferrets without urinary tract disorders admitted to veterinary teaching hospitals in the United States between January 1, 1992, and December 31, 2009. They were identified by searching the records of the Veterinary Medical Database, [b] which is responsible for compiling patients' encounter data from nearly all North American veterinary medical colleges. Because preliminary evaluation indicated that cystine uroliths were not detected in ferrets < 6 months of age, control ferrets of this age were excluded from the study.

Urolith analysis—The mineral composition of the uroliths was determined by optical crystallography and, when necessary, by infrared spectroscopy.[42] Uroliths containing at least 70% of a single mineral with lesser quantities of other minerals were classified as that mineral type. Uroliths containing nuclei and shells of at least 70% of two or more different mineral types were classified as compound. Uroliths containing < 70% of a single mineral component and without a nucleus or shell were classified as mixed.

Optical crystallography was performed using polarized microscope. In this method trained technicians removed specific crystals from several areas within the sectioned urolith with the aid of a stereomicroscope. The crystals were immersed in oils of known refractive index, and were viewed through a polarizing microscope. Based on the refractive index and crystalline properties, determinations of mineral compositions were made. The polarizing microscopes used in the study included the Olympus BX50 and the Nikon Eclipse 50i polarizing microscopes.

Infrared spectroscopy was used to detect the vibrational characteristics of chemical functional groups. Diffused reflectance infrared Fourier transforms spectroscopy (DRIFTS) and /or Attenuated total reflection (ATR) allowed for determination of composition with mineral sample preparation. The resulting spectrum from either technique was compared with a special library of known compounds to determine the composition of the sample. Nicolet iS10 Infrared Spectroscope from the Thermo-Scientific Corporation was used in this study. There were situations were additional quantative techniques were used when necessary to identify mineral composition of some the cases. In these scenarios we utilized x-ray crystallography and electron dispersive spectroscopy which was available through collaborating laboratories at the University of Minnesota and elsewhere as was necessary.

History—Information about breed**,** age, sex, reproduction status, location of the uroliths within the urinary tract, season of detection, and geographic location for each ferret was obtained from the submission forms (urolith analysis request form). Ferrets are not classified according to breed but are commonly differentiated by their coat colors, [50]although all are considered domestic ferrets; therefore, evaluation of possible associations with breed were not possible.

Anatomic location of cystine uroliths—Anatomic location of the uroliths was divided into 2 categories. Nephroliths and ureteroliths were classified as uroliths of the upper portion of the urinary tract. Uroliths that were retrieved from the urinary bladder or urethra and uroliths that were voided were classified as uroliths of the lower portion of the urinary tract. Uroliths retrieved from upper and lower portions of the urinary tract were classified as both. When the location of the uroliths was not provided, the location was classified as unknown.

Statistical analysis—Standard statistical software [44e,f,g&k,] was used to determine descriptive statistics (mean, median, and SDs) of age (i.e., 6 months to < 1 year, 1 to < 2 years, 2 to < 3 years, 4 to < 7 years, ≥ 7 years, and < 4 vs ≥ 4 years), sex, reproductive status, anatomic location of the uroliths, and the seasons of urolith submissions.

Crude and adjusted ORs and logistics regression calculated at 95% CIs by use of the Woolf method [45] to assess whether, age, sex (male or female), reproductive status (neutered or sexually intact), season[h] (fall, spring, winter, or summer), and anatomic location (lower portion of the urinary tract or upper portion of the urinary tract) were associated with the occurrence of cystine uroliths. If any expected cell frequency in a contingency table was < 5, the Fisher exact test was used.[46] Prevalence of each urolith mineral types was expressed as relative frequency compared with all the uroliths submitted for analysis to the MUC. Relative frequencies also were used to describe the age, sex, reproductive status, and anatomic location.

Reference groups for statistical analysis were arbitrarily chosen as follows: Ferrets ≥ 6 months to < 1-year-old, ≥ 4 years old, female, sexually intact, upper portion of the urinary tract, and spring season. The 18-year study was arbitrarily grouped into 4 intervals (1992 to 1996, 1997 to 2001, 2002 to 2006, and 2007 to 2009) to determine whether risk or protective factors changed over time. The Breslow-day statistic was computed to determine whether ORs were homogeneous over the 4 time intervals.[47] The Mantel-Haenszel summary of OR was computed when the results of the Breslow-Day test were not significant.

Because of the absence of continuous variables, OR values were computed via hierarchical well-formulated modeling to find the best risk model for age group, sex, reproductive status, and season. After adjusting for confounding factors and interactions, risk factors associated with urolith formation 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.[48] On the basis of recommendations by Lilienfeld and Stolley[49] we classified significant ORs between 1.1 and 1.9 and ORs between 0.5 and 0.9 as weak associations. Likewise, we interpreted significant ORs > 2 (i.e., risk) and ORs < 0.5 (i.e., protective) as clinically (biologically) important. All analyses were performed with standard software.[44, e] Results were considered significant at values of $P < 0.05$.

RESULTS

Urolith analysis—Of the 435 ferret uroliths, 70 (16%) were cystine uroliths (**Table 1**). All ferrets with cystine uroliths resided in the United States. Among all uroliths, sterile struvite (magnesium ammonium phosphate $6H_2O$) uroliths were the most common by mineral type (227[64%]). Calcium oxalate was identified as the primary component in 11% of the uroliths.

Age—The minimum age of ferrets at the time cystine uroliths were retrieved was 6 months (the arbitrary lower limit) and the maximum was 9 years. Mean ± SD age of ferrets with cystine uroliths was 4.1 ± 1.5 years and the median age was 4 years. Cystine uroliths were found most commonly in the 2 to < 4 years' age group (51%) followed by the 4 to < 7 years' age group (21%). Taking ≥ 6 months to < 1 year as the baseline control group for comparison, 1 to < 2-year-old ferrets were 3.9 times as likely to develop cystine uroliths than were ferrets in the control group (**Table 2**). Ferrets 2 to < 3 years old were 4 times as likely to develop cystine uroliths as were ferrets ≥ 6 months to < 1-year-old. Likewise, 4- to < 7-year-old ferrets were 3 times more likely to develop cystine uroliths than were ferrets in the control group.

For the Breslow-Day test, it was necessary to allocate ferrets into 2 age groups: those < 4 years old and those that were ≥ 4 years old. Ferrets ≥ 4 years old were 4.7 times as likely to develop cystine uroliths as were ferrets < 4 years old. Age was therefore a significant factor in the occurrence of cystine uroliths in ferrets.

Sex—Cystine uroliths in ferrets occurred more frequently in males (n = 54 [77%]) than in females (16 [23%]). Of the 70 ferrets with cystine uroliths for which sex was recorded, males were 2.5 times as likely to develop cystine uroliths as were females (**Table 2**).

Reproductive status—Sixty-five (93%) of the ferrets with cystine uroliths were neutered, whereas 5 (7%) were sexually intact. Of the 70 ferrets with cystine uroliths for which the reproductive status was recorded at the time of collection, neutered ferrets were 3.1 times as likely to develop cystine uroliths as were sexually intact ferrets (**Table 2**).

Anatomic location—Of the 70 cystine uroliths, 67 (96%) were retrieved from the lower portion of the urinary tract; 56 (84%) came from urethra, and 11 (16%) came from the bladder. Three (4%) of the 70 uroliths were voided; therefore, their original anatomic locations were not known. The cystine uroliths were 5.4 times as likely to be retrieved from the lower portions of the urinary tract (**Table 2**).

Seasonal distribution—Of the 69 cystine uroliths for which the season of collection was recorded at the time they were retrieved, 17 (24%) were recorded in the fall, 12 (17%) in the spring, 22 (31%) in the summer, and 18 (26%) in the winter. Season of collection was not recorded for 1 urolith. With spring used as a reference season, probability values observed for fall (P = 0.78), winter (P = 0.73) and summer (P = 0.74) were not significant (**Table 2**).

Geographic distribution and submission rate—Of 56 ferrets with cystine uroliths for which the geographic locations in the United States were recorded, 10 (18%) were from the Northeast, 5 (9%) were from West, 1 (2%) were from Southwest, 27 (48%) were from Southeast, and 13 (23%) were from Midwest. Fourteen cases were excluded on the basis of incomplete recording of location. The number of ferret cystine urolith submissions progressively increased from 4 in the period 1992 to 1997, to 19 in the period 1998 to 2003, to 47 in the period 2004 to 2009.

Between January 2010 and September 2012, uroliths from an additional 69 ferrets were submitted to the Minnesota Urolith Center for analysis. Of these 69 uroliths, 44 (64%) were cystine uroliths, 12 (17%) were struvite uroliths, and 6 (9%) were calcium oxalate uroliths, and 3 (4%) were ammonium nitrate uroliths. In addition, there was 1 urolith of mixed composition, 1 compound urolith, 1 urolith composed of miscellaneous materials, and 1 urolith composed of inspissated blood.

Of the 41 ferrets with cystine uroliths and with reproductive status recorded, 36 of 41 (88%) were neutered males and 5 of 41 (12%) were neutered females. For 44 of the ferrets for which age was available, mean ± SD age was 2.99 ± 1.5 years (range, 9.5 months to 6 years), with 8 (18%) being < 2 years old, 25 (57%) being 2 to < 4 years, and 11 (25%) being ≥ 4 years old.

Cystine urolith composition— Quantitative analysis of 70 cystine uroliths revealed that all were composed of 100% cystine. The uroliths were ovoid and smooth, light yellow to tan, and ranged from 0.5mm to several centimeters in diameter (**Figure 1**). The number of uroliths in each ferret varied from 1 to > 100.

Breed—Unlike cats and dogs, ferrets are not classified according to breed. They are differentiated by their coat colors[50] Ferrets with different coat colors are often grouped together into 1 category designated as domestic ferrets. We were unable to classify ferrets by breed or coat color.

DISCUSSION

Cystine (also termed dicysteine) is a nonessential sulfur-containing amino acid that is normally present in low concentration in plasma. In healthy dogs and humans, cystine is freely filtered by glomeruli.[69]Almost all the filtered cystine is then actively reabsorbed by the proximal tubules. Cystinuria in humans, dogs and cats is typically characterized by impaired renal tubular reabsorption of cystine and occasionally other amino acids.[69-72] Cystine is the least soluble of the 4 dibasic amino acids (cystine, ornithine, lysine, arginine, [aka as the acronym cola]) commonly found in urine of cystinuric patients and is an important risk factor for urolithiasis. The solubility of cystine is pH dependent; it is less soluble in acid than in alkaline urine. Concentrated urine and an acidic urine pH increase the insolubility of cystine, whereas alkaline urine increases the solubility of cystine uroliths.[70] Because studies in humans[71] and dogs[70] suggest a familial pattern of inheritance of this disease caused by genetic defects, it is likely that ferret cystinuria is also associated with genetic defects. However, further studies are needed to confirm this possibility.

In the present study, the mean age of ferrets at the time of detection of cystine uroliths was approximately 4 years. This was surprising, as one might expect an earlier onset of clinical manifestations of a probable genetic disorder, with the life span of ferrets being reported as 5 to 11 years.[58]

Cystine uroliths were submitted from male ferrets more often (77%) than from females (23%). Unlike in cat in which cystine uroliths occur in males and females with equal frequency, male ferrets were approximately 2.5 times as likely to develop cystine uroliths as were female ferrets (**Table 2**). This difference is likely related to the unique J-shaped anatomy of the distal portion of the urethra of male ferrets, which is also smaller in diameter than the proximal portion of the urethra.[58-59]This likely predisposes male ferrets to partial or total obstruction of the urethral lumen with uroliths.

It was observed that a higher proportion of neutered male and female ferrets (85%) had cystine uroliths, compared with sexually intact ferrets (15%). Neutered ferrets were approximately 3.1 times as likely to develop cystine uroliths as were sexually intact ferrets (**Table 2**). However, we do not think that there was an association between reproductive status and development of cystine uroliths because the control population had a similar sex distribution. Also, results of the study did not suggest that cystine uroliths may be influenced by season of submission because no significant differences were observed among different seasons. The number of ferret cystine urolith submissions progressively increased from 4 in the period 1992 to 1997, to 19 in the period 1998 to 2003, to 47 in the period 2004 to 2009, but the study was not designed to determine a cause for this increase in submission rate.

Cystine was the second most prevalent type of urolith in ferrets of this case series. The genetics, biological behavior, and treatment of cystine urolithiasis have been extensively reported in dogs and humans. [70-71] Other investigators have reported that cystine uroliths rarely occur in ferrets.[51, 70-74] However, cystine uroliths were observed in 70 of 435 (16%) of ferret uroliths submitted during the 18-year period of the present study. In contrast, cystine uroliths were found in 102 of 114,935 (0.09%) of cat uroliths submitted to the Minnesota Urolith Center and 4,310 of 429,471 (1%) of dog uroliths submitted to the Minnesota Urolith Center between 1992 and 2009. [60]

Apparently, the biological behavior of cystine urolithiasis in ferrets has not been studied, as we could only find empirical accounts of it in the literature. The biological behavior of cystine uroliths in 3 cats has been reported.[60]The uroliths were detected when the cats were < 1 year of age. The cats died of complications associated with chronic progressive renal failure when they were 5 to 7 years of age.

Although a familial basis for cystine urolithiasis has been well documented in dogs and humans, a genetic predisposition has not yet been proven in ferrets.[70-71]The observation that cystine uroliths are associated with a familial cause in other species, and the relatively large percentage of cystine uroliths observed in ferrets compared with the percentage in other species, suggests that the parent stock of the ferrets in the present study may have been inbred. The observations regarding the uroliths submitted between January 2010 and September 2012 provide further support to the suggestion that there is a familial predisposition for cystine urolithiasis in domestic ferrets and that domestic ferret in the United States may be highly inbred. Additional studies are needed to evaluate these hypotheses.

Biases and limitations: (1) Since our data was obtained from ferret's urolith submissions to the MUC, it is not likely a complete representative of all urolith submissions of all breeds, age, sex, season and geographic location of all ferret populations.

(2) The urethras of male ferrets are longer and narrower than females. Also the males have J-shaped penises which are absent in females. All these features may have contributed to more clinical manifestations of urethral obstruction in males compared to females and may have therefore influenced their selection as cases.

(3) Since the Minnesota Urolith Center uroliths analyses are performed at no cost to veterinarians this may have influenced the rate of ferret urolith submissions to the center.

(4) Because the sample size in this study was not very large we are not able to apply the conclusions obtained from our findings to the whole ferrets' population.

(5) Uroliths obtained from male ferrets in this study were retrieved from the lower portion of the urinary tract. Clinical signs are more likely to be observed when uroliths are located in the lower urinary tract than from the upper urinary tract. This may have constituted a form of bias in this study leading to higher number of lower urinary tract uroliths observed in this study.

(6) This study may have included more clinical cases, and may not have included subclinical cases, asymptomatic cases or cases that may have been euthanized due to urinary tract obstructions; as a result, this may have limited the use of results from this study to fully represent the population of all ferrets with uroliths because of this form of bias. (7) Higher prevalence of uroliths may have been observed in pet ferrets because of the increase in the trend of keeping them as pets. As pets, they may have received more veterinary care when sick compared with ferrets kept for other purposes. This trend may have influenced the submission of uroliths from this group of ferrets compared with others (ferrets used for other purposes).

Table 1—Mineral composition of 435 ferret uroliths evaluated at the MUC from 1992 to 2009.

Mineral composition *		**Number of uroliths**	**%**
Magnesium ammonium phosphate 6H20	70-100%	**277**	64
Magnesium hydrogen phosphate 3H20	100%	**1**	< 1
Cystine	100%	**70**	16
Calcium oxalate monohydrate and dihydrate	70-100%	**50**	11
Calcium phosphate	70-100%	3	1
Calcium carbonate	70-99%	**1**	< 1
Ammonium urate	70-100%	**8**	1.8
Miscellaneous material	70-100%	**6**	1.4
Mixed †	70-100%	**8**	<1.8
Compound ‡	70-100%	**6**	<1.4
Silica	100%	**1**	<1
Other	70-100%	**4**	<1
TOTAL		435	100

*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 1or more surrounding layers of a different mineral type.

Table 2—Crude and adjusted ORs and logistic regression analysis of variables associated with cystine uroliths in 70 ferrets.

Variables	Description	Crude OR	95% CI	Adjusted OR	95% CI	*P* value
Age	(1 y - < 2 y) vs (≥ 6 mo - 1 y)	4.1	3.1-12.6	3.9	2.7-14.4	< 0.001
	(2 y -< 3 y) vs (≥ 6 mo - 1 y)	4.8	4.6-11.2	4.1	3.2-11.1	< 0.001
	(4 y - < 7 y) vs (≥ 6 mo - 1 y)	3.9	2.8- 4.6	3.2	2.7 - 4.8	< 0.001
	≥ 4 y vs (≥ 6 mo - 1 y)	1.2	1.1-2.3	1.1	0.02-2.9	0.80
	≤ 4 y vs ≥ 4 y	5.2	4.6-15.3	4.7	4.5 – 18.9	< 0.001
Sex	Male vs female	2.7	2.3- 12.6	2.5	2.3 – 15.2	< 0.001
Reproductive status	Neutered vs sexually intact	3.6	3.2-8.6	3.1	2.4 – 9.2	< 0.001
Anatomic location	Lower vs upper portion	5.3	4.8-12.5	5.4	4.6 – 16.4	< 0.001
Season	Fall vs spring	1.1	0.9-3.3	0.9	0.2 – 2.8	0.78
	Summer vs spring	1.3	1.2-4.6	0.8	0.2 – 4.6	0.73
	Winter vs spring	1.0	0.3-2.3	0.9	0.2 – 11.3	0.74

Figure 1—Photograph of typical cystine uroliths from the urinary bladder of a neutered, 6-year-old male ferret. Scale indicates centimeters.

ADDENDUM

Between January 2010 and September 2012, uroliths from additional 69 ferrets were submitted to the Minnesota Uroliths Center for analysis. Of these 69 uroliths, 44 (64%) were cystine uroliths, 12 (17%) were struvite uroliths, 6 (9%) were calcium oxalate uroliths, and 3 (4%) were ammonium urate. In addition, there was 1 urolith of mixed composition, 1 compound urolith, 1 urolith composed of miscellaneous material, and 1 urolith composed of inspissated blood.

Of 41 ferrets with cystine uroliths in which their reproductive status was recorded, 36 of 41 (88%) were neutered males and 5 of 41 (12%) were neutered females.

For 44 ferrets for which age was available, Mean ± SD age was 2.99 ± 1.5 years (range, 9.5 months to 6 years), with 8 (18%) < 2 years old, 25 (57%) 2 to < 4 years old, and 11 (25%) ≥ 4 years old.

These observations provide further support to our suggestion that there is a familial predisposition for cystine urolithiasis in domestic ferrets, and that domestic ferrets in this country may be highly inbred. Additional studies are urgently needed to evaluate these hypotheses.

The surge of cystinuria in ferrets has not abated because between January 1, 2013 and December 31, 2015, an additional 379 uroliths from additional 379 ferrets were analyzed at the MUC. Of these 350 of 379 (92%) uroliths were cystine, 22 of 379 (6%) uroliths were struvite and the remaining uroliths which were 7 of 379 (2%) comprised other uroliths outside cystine and struvite. Cystinuria may be genetic in ferrets as in dogs, cats and humans.

CHAPTER 4

Risk factors for calcium carbonate urolithiasis in goats.

SUMMARY

The objective of this study was to identify demographic or signalment factors associated with calcium carbonate urolith formation in goats. Medical records of the Minnesota Urolith Center were reviewed to identify case goats for which samples were submitted between January 1, 1984, and December 31, 2012. Controls were goats without urinary tract infections evaluated at US veterinary teaching hospitals in the same time period and were identified by searching the Veterinary Medical Database records. Breed, age, sex, reproductive status, geographic location of goats, season, and anatomic location of collected uroliths were analyzed to identify risk or protective factors associated with calcium carbonate urolithiasis. The Mean ± SD age of goats with $CaCO_3$ urolith was 3.8 ± 1.8 years and range (0.3 to 14 years). Nigerian dwarf goats had higher odds of developing calcium carbonate uroliths than did Pygmy goats (reference group). Several breeds had lower odds of this finding, compared to Pygmy goats; odds were lowest for mixed, Anglo-Nubian, and Toggenburg breeds. Breeds of African origin (Pygmy, Nigerian Dwarf, and Boer) comprised 146 of 275 (53%) case goats with data available. Goats of African descent had a higher risk of developing $CaCO_3$ uroliths than did goats of non-African descent (reference group). Male goats had higher odds for $CaCO_3$ uroliths compared to females. Neutered goats had higher odds for $CaCO_3$ uroliths compared with sexually intact goats. Age category, geographic location, and season were associated with detection of calcium carbonate uroliths. Goats with calcium carbonate uroliths were typically neutered males, > 1 year of age, and of African descent.

This study identified risk and protective factors associated with $CaCO_3$ urolithiasis in goats; however, these associations do not allow conclusions regarding cause-and-effect relationships.

INTRODUCTION

Goats are becoming increasingly popular as pets in the United States.[25-27] Leading this trend of popular pet goats are breeds of African descent. [25-27] The affectionate and docile nature of goats of African origin likely has contributed to their popularity as pets in the United States.[25-27] As of January 2014, the population of goats in the United States was estimated to be 2.3 million by the Agricultural Statistics Board.[75] Goats, along with being pets, are used for food, fiber, weed control, youth development organization projects, animal cell culture production, pack animal activities, and competition in livestock shows.[75] However, not much is known about changes in diets and natural habits of these breeds of African goats in their native continent.[76]

As the population of pet goats increases, uroliths are being detected with increased frequency. One explanation may be that pet goats may receive more intensive medical attention than more traditional livestock. It is also possible that pet goats are more likely to be housed individually or kept at residences because of their small size and therefore are under closer observation than animals not kept as pets. Knowledge of the various mineral compositions of uroliths affecting goats and risk factors for urolith formation are needed to develop effective diagnostic, therapeutic, and preventive strategies.

Between 1981 and 2007, 941 urolith submissions were sent to the Minnesota Urolith Center (MUC) from 19 ruminant species, and the prevalence of calcium carbonate uroliths was highest in moose, wildebeest, and goat. During this time period, 42% of goat uroliths submitted to this facility were composed of calcium carbonate.[8] Urinary tract obstructions resulting from complications of urolithiasis in goats have been observed almost exclusively in males. In male goats, the distal penile urethra forms a lengthy, narrow, and tortuous urethral process that is a common site for uroliths to lodge.[26] Published reobstruction rates for goats with obstructive urolithiasis, even after prompt and aggressive treatment, range from 8 of 28 (29%) to 6 of 20 (30%).[4, 27]

A review of uncontrolled clinical studies of obstructive urolithiasis in goats resulted in the observation that the time to reobstruction following initial treatment ranges from 1 to 8 months.[27] Our empirical observations suggest that the rate of recurrence may be minimized by modifying dietary ingredients, with the goals of reducing lithogenic minerals and increasing urine volume.[77]

Risk can be defined as the probability of disease developing in an individual during a specific interval.[77] As clinicians, we think of risk factors and protective factors as events that will affect the biological behavior of diseases such as urolithiasis.[77] Evaluation of risk and protective factors through epidemiological studies may be useful in the development of recommendations for the prevention and control of urolithiasis.[78]

The purpose of the study reported here was to determine the predominant mineral type of uroliths collected from goats and submitted to the MUC and to characterize risk and protective factors associated with urolithiasis in this species. In addition, we sought to assess associations of potential risk factors (breed, age, sex, reproductive status, anatomic location within the urinary tract, season of sample collection, and geographic location) with calcium carbonate urolith detection. We hypothesized that breed, age, sex, reproductive status, geographic location, and season would be risk factors associated with calcium carbonate urolith detection in goats.

MATERIALS AND METHODS

Identification of cases and controls and medical records review—Medical records of the MUC[a] were reviewed to identify goats for which urolith samples were submitted by veterinarians within the United States between January 1, 1984 and December 31, 2012. The mineral composition of the uroliths was determined by optical crystallography[42, 79] and, when necessary, by infrared spectroscopy.[42] Case animals were identified as goats that had uroliths consisting of ≥ 70% calcium carbonate. Animals with compound uroliths (i.e., those containing nuclei, stone, shell, or surface crystal layers with ≥ 2 layers with < 70% different mineral types) were excluded. Preliminary evaluation of case series data indicated that uroliths were not detected in goats < 3 months of age, so goats of this age in the control group were censored.

Goats without urinary tract disorders (control goats) evaluated at the veterinary teaching hospitals in the United States between January 1, 1984, and December 31, 2012, were identified by searching the records of the Veterinary Medical Database.[b] Only animals that had urinalysis performed and had no urinary tract diseases were selected as controls.

The following information was retrieved from urolith submission records of each case animal and medical records of controls when applicable: breed, age, sex, reproductive status (neutered or sexually intact), state of residence, date of urolith submission, anatomic location of the urolith (upper urinary tract [kidneys and ureters], lower urinary tract [bladder, urethra, and voided samples], upper and lower urinary tract, or unknown), and diet (first and second components listed on the submission records). Distinction between the Anglo-Nubian and Nubian goat breeds could not be consistently determined from medical records of goats with uroliths submitted to the MUC. Therefore, goats of these 2 breeds were grouped together in the present study. Geographic regions (Southeast, Southwest, Midwest, Northeast, or West) were determined on the basis of state of residence according to classification established by the National Geographic education division[j]. Seasons[i] of sample collection were classified as winter (December, January, February), spring (March, April, May), summer (June, July, August), and fall (September, October, November).

Methods of quantitative urolith analysis – Uroliths for the cases in this study were analyzed by optical crystallography and infra-red spectroscopy which were both quantitative urolith analysis methods. In this study only uroliths from cases that were ≥ 70% of the primary mineral were included. Optical crystallography was performed using polarized microscope. In this method trained technicians removed specific crystals from several areas within the sectioned urolith with the aid of a stereomicroscope. The crystals were immersed in oils of known refractive index, and were viewed through a polarizing microscope. Based on the refractive index and crystalline properties, determinations of mineral compositions were made. The polarizing microscopes used in the study included the Olympus BX50 and the Nikon Eclipse 50i polarizing microscopes.

Infrared spectroscopy was used to detect the vibrational characteristics of chemical functional groups. Diffused reflectance infrared Fourier transforms spectroscopy (DRIFTS) and /or Attenuated total reflection (ATR) allowed for determination of composition with mineral sample preparation. The resulting spectrum from either technique was compared with a special library of known compounds to determine the composition of the sample. Nicolet iS10 Infrared Spectroscope from the Thermo-Scientific Corporation was used in this study.

There were situations where additional quantitative techniques were used when necessary to identify mineral composition of some of the cases. In these scenarios we utilized x-ray crystallography and electron dispersive spectroscopy which was available through collaborating laboratories at the University of Minnesota and elsewhere as was necessary.

Statistical analysis—Standard statistical software[44, e, f, g, &k] was used to determine descriptive statistics (mean, median, and SDs) of breed, age, sex, reproductive status, geographic location of goats, season of urolith submission, and anatomic location of the uroliths within the urinary tract for case animals. Odds ratios (OR) and logistic regression were calculated at 95% confidence intervals (CI) by use of the Woolf method[45] to assess whether breed, age, sex, reproductive status, season, and geographic location were associated with the presence of calcium carbonate uroliths. If any expected cell frequency in a contingency table was < 5, the Fisher exact test was used.[46]

Breeds were further categorized as African (Pygmy, Nigerian Dwarf, and Boer) or non-African (all others) in origin. For analysis of age effects, goats were divided into 6 age groups: < 1 year, 1 to < 2 years, 2 to < 4 years, 4 to < 7 years, 7 to < 10 years, and ≥ 10 years. Data for goats < 4 years of age were also compared with those for goats' ≥ 4 years old. Referents for the variables (determined on the basis of breaks in the data and mean age) for determination of ORs were as follows: breed, mixed; breed origin, non-

African; age, < 1 year; sex, female; reproductive status, sexually intact; season, spring; and geographic region, Southwest.

Prevalence of calcium carbonate uroliths was expressed as the relative frequency of all goat uroliths submitted for analysis to the MUC. Relative frequencies also were used to describe the breed, age, sex, reproductive status, and geographic location of goats, as well as season of collection and anatomic location of uroliths. Logistic regression was computed to determine whether the yearly percentage of goats with calcium carbonate uroliths of Africa and non-African origins increased or decreased over the study period. The 28-year study was grouped into 2 intervals (1984 through 1998 and 1999 through 2012) to determine whether risk or protective factors changed over time.

The Breslow-Day statistic was computed to determine whether ORs were homogenous over the 2 time intervals.[47] The Mantel-Haenszel summary of ORs was computed when the results of the Breslow-Day test were not significant. Values of $P < 0.05$ were considered significant. Because of the absence of continuous variables, crude ORs were computed by means of hierarchic well-formulated modeling to find the best risk model for the categorical variables of breed, age, sex, reproductive status, geographic location, season, and anatomic location. After adjusting for age, sex, and reproductive status as confounding factors and interactions, risk factors associated with urolith formation were determined from the best model.

Odds ratio estimates were considered significantly different from 1 if the 95% CI did not encompass 1.0.[48] On the basis of recommendations by Lilienfeld and Stolley, [49] we classified associations with significant ORs between 1.1 and 1.9 or between 0.5 and 0.9 as weak. Significant ORs > 2 (indicating a risk factor) and those < 0.5 (suggesting a protective effect) were interpreted as clinically (biologically) important. All analyses were performed with standard software.[44, e, f, g, &k] Results were considered as significant at values of $P < 0.05$.

RESULTS

Uroliths from 832 goats were submitted to the Minnesota Urolith Center for analysis during the study period. Of these, uroliths containing ≥ 70% calcium carbonate were the most common (n = 364 [44%]). After censoring submissions from outside the United States (n = 10), calcium carbonate content of the remaining 354 samples from 354 case goats was as follows: 100%, 337 (95%) samples; 90% to 99%, 8 (2%) samples; and ≥ 70% to 89%, 9 (3%) samples. Calcium carbonate uroliths retrieved from goats were typically spherical and smooth **(Figure 2)**. They were light yellow to golden brown in color and ranged from < 1 mm to > 1 cm in diameter. The number of uroliths submitted from each case goat varied.

Breed—Case goats included 17 breeds and control goats included 11 breeds. Six breeds were excluded from breed-related analysis because of a lack of control animals; these included Pygora (n = 5 case goats), French Alpine (3), Fainting Goat (2), and British Alpine, Cashmere, and Norwegian (1 each). Breed data for 79 of 354 (22%) case and 124 of 16,366 (0.8%) control animals were not provided at the time of urolith submission. The breed with the highest odds of developing calcium carbonate uroliths, compared to Pygmy goats, was Nigerian Dwarf ($P < 0.001$; **Table 1**). Breeds with the lowest risk of developing calcium carbonate uroliths, compared to Pygmy goats, included mixed, Anglo-Nubian or Nubian, and Toggenburg. Of the 275 submissions where breed was recorded, 147 (53%) and 128 (47%) were breeds of African and non-African origins, respectively. Goats of African descent had significantly (P < 0.001) higher odds of developing calcium carbonate uroliths than did the referent group (OR, 3.85). From 1984 through 1998, case goats of African descent constituted 17 of 36 (47%) goats with calcium carbonate uroliths. Goat of non-African descent constituted 19 of 36 (53%) of this group. During the same period, 1,579 of 11,205 (14%) controls goats were of African descent, and the remaining 9,626 (86%) were of breeds with other origins. From 1999 through 2012, 130 (54%) and 109 (46%) of 239 case goats were of African and non-African descent, respectively. Among 5,037 control goats seen during this period, breeds of 1,816 (36%) and 3,221 (64%) were of African and non-African origin, respectively. Breed origin data were unavailable for 79 case and 124 control goats seen during 1999 through 2012.

Age—Mean ± SD age of 296 case goats for which data were available was 3.8 ± 1.8 years (range, 3.8 months to 14 years). One hundred and sixty-five (56%) of these goats were < 4 years and 131 (44%) were ≥ 4 years of age. The odds of developing $CaCO_3$ uroliths were significantly greater for goats in all other age groups, compared with odds for the referent group of < 1 year of age **(Table 2)**. When combined age groups were evaluated, goats that were < 4 years old were significantly less likely (adjusted OR, 0.26) to develop calcium carbonate uroliths than goats ≥ 4 years old (referent group).

Sex and reproductive status—Of 354 case goats, sex was recorded for 346 (98%). Of the 346, 343 (99%) were males and 3 (1%) were females. Males were approximately 196 times as likely to develop calcium carbonate uroliths as were females (referent group;

Table2). Reproductive status for 346 of 354 (98%) case goats was recorded; 308 (89%) of these were neutered and 38 (11%) were sexually intact. A higher proportion of neutered male goats (307/343[90%] had calcium carbonate uroliths, compared with sexually intact males (36/343 [10 %]; $P < 0.001$). Neutered goats were approximately 56 times as likely to have calcium carbonate uroliths as sexually intact goats (**Table 2**).

Anatomic location—Sites from which the uroliths were collected were reported for 342 case animals and unknown for 12. Three hundred and thirty-six of 342 (98%) goats had samples obtained from the lower portions of the urinary tract (urinary bladder, urethra, voided uroliths, or some combination of these). Uroliths from 3 (1%) goats included material collected from both the upper (kidney or ureter) and lower urinary tracts. Submitted uroliths were from the bladder only for 93 (27%) goats, and from the urethra only for 137 (40%) goats. Submission of uroliths from the kidney (n = 1) or ureter alone (2) was uncommon.

Seasonal distribution—Collection and submission of uroliths for the 354 case goats took place in fall (n = 147 [42%]), summer (87 [25%]), winter (78 [22%]), and spring (42 [12%]). Calcium carbonate uroliths were significantly ($P < 0.001$ for all comparisons) more likely to be collected and submitted in fall, summer, and winter than in spring (**Table 2**).

Geographic distribution—Ninety-four of 354 (27%) case goats were from the Midwest, 39 (11%) were from the Northeast, 52 (15%) were from the Southeast, 15 (4%) were from the Southwest, and 154 (44%) were from the West geographic zones. The frequency of submission of calcium carbonate was approximately 2 times as great in the Southeast as in the Southwest (*P* = 0.007). Likewise, the frequency of submission of calcium carbonate uroliths in the West and Northeast was approximately 12 times that in the Southwest (**Table 2**; ***P* = < 0.001**).

Diet—The primary types of diet were recorded for 311 of 354 (88%) case goats. One hundred and seven of the 311 (34%) goats were fed grass or kept on pasture, 74 (24%) were fed grass hay, 37 (12%) were fed alfalfa hay, 55 (18%) were fed commercial foods (type not specified on the submission forms), 26 (8%) were fed whole grains (e.g., corn or wheat), and 12 (4%) were fed hay and pelleted grain mixture (pelleted grains blended with vitamins and minerals). More goats fed on pasture were most at risk; while goats fed on hay and pelleted grain mixture were at least risk (**Table 2 P= < 0.0001**).

DISCUSSION

The results of the present study support our hypothesis that multiple risk factors such as breed, age, sex, reproductive status, geographic location, and season of the year play a role in the development of calcium carbonate uroliths. Our results are in agreement with reports by other investigators.[3, 37] However; those studies [3, 37] were performed without controls. Instead, generalities they observed were based on uncontrolled empirical observations.

As theorized in urolith formation in other species, it is probable that when several risk factors occur together, their individual effects may be enhanced. Thus, each risk factor or protective factor may play either a limited or a major role in the development or prevention of calcium carbonate uroliths.[77] However, case-control studies usually cannot demonstrate that risk factors are the cause of a disease. The case animals in the present study were likely goats with clinical signs and may not have included subclinical cases, such as goats that voided uroliths uneventfully, or those whose uroliths were not collected and submitted to the MUC for analysis for various reasons (e.g. goats that died as a result of urinary tract obstruction or goats that were euthanized because of complications). As a result of these limitations, this study may not have fully represented the population of all goats with calcium carbonate uroliths.

Goat breeds of African descent (Pygmy, Nigerian Dwarf, and Boer) were overrepresented among those with calcium carbonate uroliths (i.e., case animals) in our study. This higher prevalence of calcium carbonate uroliths in goat breeds of African descent may be associated with a trend of keeping them as pets. As pets, they may be more likely to receive veterinary care when sick, compared with animals kept for other purposes. The number of goats with calcium carbonate uroliths in this study may reflect an increased awareness that uroliths can cause urethral obstruction and increased use of diagnostic methods to detect uroliths such as survey radiography and abdominal ultrasonography in the latter half of the study period. This may also be associated with an increased awareness of the availability of complimentary urolith analysis that influenced submissions made during these 2 periods (1984 through 1998 and 1999 through 2012) [a]

The results of our study indicate that goat breeds of African origin were at increased risk for calcium carbonate urolithiasis, compared with others breeds (i.e., those of non-African origin). Familial predisposition for urolithiasis has been well documented for some mineral types.[80] For example, genetic mutations causing cystine urolithiasis have been identified in dogs [70] and humans.[71]To the authors' knowledge a genetic predisposition for calcium carbonate uroliths in goats has not been explored. In our study, the observation that calcium carbonate uroliths more commonly affected goats of African descent, compared with other breeds, may suggest genetic predisposition of these breeds to calcium carbonate urolithiasis. Prospective studies evaluating the effects of diet, housing, body condition score, and other potential risk factors in addition to breed are necessary to further investigate a potential predisposition to this type of urolithiasis.

In our study, age was significantly associated with development of calcium carbonate uroliths in goats, with all age groups having significantly higher odds of this finding, compared with the < 1-year-old category. Epidemiological data derived from studies [55-57, 81] of dogs, cats, and humans have suggested that advancing age is a risk factor for urolithiasis.

Our results also indicated that calcium carbonate uroliths almost exclusively affected males (343/346 [99%]), compared with females (3 [1%]). The adjusted OR for this finding in males, relative to females, was 196.36 (95% CI, 62.59 to 612.52). This is likely related to the fact that male urethras are much longer and narrower than those of females.[82]As urocystoliths pass into the distal end of the male urethra (urethral process); obstructive urolithiasis is a likely consequence. The S-shaped sigmoid flexure located just caudodorsal to the testes is another potential site for uroliths to lodge. It is likely that calcium carbonate uroliths are not commonly detected in female goats because the wider and shorter urethra allows uroliths to be passed in the urine stream before they can cause clinical signs.

A higher proportion of case goats (308/346 [89%]) were neutered than were sexually intact (38 [11%]) and odds of this finding were significantly greater for neutered goats (OR, 56.48; 95% CI, 39.43 to 78.95). Given that only 1 of the 3 female case animals in this study was neutered, this statement may most appropriately be applied to the males only. This observation suggests that neutering may be a risk factor for urolithiasis. One group of investigators generated convincing evidence that early neutering may result in underdevelopment of the urethra and thus predispose adult goats to urethral obstruction.[83]The same group of investigators showed that neutering reduces production of testosterone, which normally aids in the development of urethral lumen.[83] A reduced circulating testosterone concentration is also believed to diminish normal preputial-penile attachments.[83] Because the ages at which case goats were neutered was not provided, we were unable to determine whether there was a significant association between urolithiasis and early neutering.

Three hundred thirty-six of 342 (98%) case animals in this study had calcium carbonate uroliths retrieved from the lower urinary tract. The predominant location of uroliths in the lower urinary tract of other domestic animals has been described.[84]In our experience in other species, the development of uroliths in the upper urinary tract is likely underreported, because survey radiography and ultrasonography are often omitted during initial evaluation of animals with clinical signs of urolithiasis. Clinical signs caused by uroliths in the lower portions of the urinary tract (increased voiding frequency, straining to void, and dribbling urine) are more likely to be observed than are clinical signs caused by uroliths located in the upper portions of the urinary tract (polyuria and hematuria without obvious signs of pain, which may go unrecognized). In the case animals of the present report, uroliths were more commonly retrieved from the urethra only (137/342 [40%]) than from the bladder only (93 [27%]). These localizations are important when considering dietary modification for dissolution of uroliths. It has been recommended that attempts to dissolve urethral uroliths by dietary modification be avoided.[82] Uroliths lodged in the ureters or urethra cannot be dissolved by medical protocols because urolith dissolution requires sustained contact with urine that has been modified and therefore is undersaturated with lithogenic mineral.[84]

In our study, goats residing in the West, and Northeast, regions of the United States had significantly higher odds of developing calcium carbonate uroliths than did goats in the Southwest. These observations indicate that geographic location may be a risk factor for urolithiasis. Geographic prevalence of urolithiasis has been reported in dogs and humans.[85-86]Our study was not designed to determine the reason for the variations among geographic locations. Further research is needed to assess the factors that contribute to such differences.

Calcium carbonate uroliths were significantly ($P < 0.001$ for all comparisons) more likely to be collected and submitted for case goats during the summer, fall, and winter than in spring. The association between season and presence of urolithiasis is in agreement with findings of other investigators, who reported that detection of urolithiasis in cats, sheep, and humans varies with season (with odds in summer, fall, and winter being greater than in spring [OR, 2.5, 5.4, and 2.9, respectively; $P < 0.001$]).[87-90] This may be partly attributable to higher seasonal temperatures observed during the summer and fall (leading to heat-induced water loss) in comparison to winter and spring,[87-90] and it is also possible that reduced water intake during the winter season could lead to increased urine concentration. We did not investigate possible associations between calcium carbonate urolithiasis and these factors.

Diet has been incriminated as important risks factor for calcium carbonate urolithiasis in goats.[90-91]In our study; we encountered a lack of detailed information regarding diet composition and variability in the reported diets fed to case goats. Lack of consistency in response to our requests for dietary history prevented us from vouching the result of the evaluation of dietary components as risk factors for this disease. To our knowledge, controlled, blinded clinical studies have not been performed to evaluate associations between most dietary factors and urolith formation in goats. Our study was also not designed to explore this factor, and we were unable to test for such associations, although it is likely that diet has a role in the pathogenesis of calcium carbonate urolithiasis.

However, during cold seasons like winter when pastures contain scanty forages grass hay and alfalfa hay are typical diets used for feeding goats. Most case goats for which diet was reported primarily received alfalfa hay or grass hay (111/311 [36%]). Alfalfa and grass hay have a high calcium-to-magnesium concentration ratio and low phosphorus concentration and are also rich in oxalate. Case goats in this study that were fed on pastures were 10 times ($P < 0.001$) as likely to be detected with calcium carbonate uroliths as those fed on hay plus pelleted grain mixtures (**Table 2**). Similarly, case goats that were fed on grass hay were 7 times ($P < 0.001$), alfalfa hay were 4 times (P-value 0.002), commercial feeds (not specified in the submission forms) were 5 times ($P < 0.001$), and whole grain were 3 times ($P = 0.011$) respectively as likely to be detected with calcium carbonate uroliths than those fed on hay plus pelleted grain mixtures. **(Table 2**) This may be related to the fact that hay plus pelleted grain feed consumption leads to increase water consumption, which will prevent urine concentration. This observation will ultimately lead to a reduction in urolith formation. Further prospective studies are needed to define the role played by each of these factors singly and in combination as they relate to calcium carbonate urolithiasis

Healthy goats normally excrete urine that is alkaline (pH range, 7.5 to 8.5). Sheep, horses, rabbits, and guinea pigs also normally excrete alkaline urine. Production of alkaline urine and calcium carbonate urolithiasis are shared characteristics among these herbivorous species. Most of the plants used to feed goats in this study (e.g., grass, grains, and alfalfa) are associated with alkaline urine production.[90-91] Although, not investigated in the present study, it is probable that alkaline urine pH is a risk factor for calcium carbonate urolithiasis in goats, and this should be investigated in future studies. Although information derived from the extensive urolith database compiled by the MUC[a] together with data from the Veterinary Medical Database[b] provides a unique resource for retrospective evaluation of the epidemiological features of this disorder, it is important to note that case-control studies and retrospective case series do not prove cause-and-effect relationships.

Additional prospective or interventional studies are needed to prove hypotheses derived from such studies. Knowledge of the predominant mineral types of uroliths in specific species, 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 goats. Modification of risk factors, where possible, may help to minimize urolith formation, dissolve existing uroliths, and minimize recurrences.

Biases and limitations. First, since our data was obtained from goat's calcium carbonate urolith submissions to the MUC, it was not a complete representative of all urolith submissions from all breed, age, sex, reproductive status, season and geographic location of all goat populations. Second, the urethras of male goats are much longer and narrower than females. Also the males have urethral process and sigmoid flexure which are absent in females. All these features may have contributed to more clinical manifestations of urethral obstruction in males compared to females and may have therefore influenced their selection as calcium carbonate urolith cases.

Since the Minnesota Urolith Center uroliths analyses are performed at no cost to veterinarians this may have influenced the rate of urolith submissions to the center. Also because the sample size in this study was not very large we were unable to apply the conclusions obtained from the findings of our study to the whole goat population with calcium carbonate urolithiasis.

Uroliths obtained from male goats in this study were retrieved mainly from the lower portion of the urinary tract. Clinical signs are more likely to be observed when uroliths are located in the lower urinary tract than from the upper urinary tract. This may have constituted a form of bias in this study leading to higher number of lower urinary tract uroliths being observed in this study compared to the upper urinary tract uroliths. Furthermore, this study may have included more clinical cases, and may not have included subclinical cases or cases that may have been euthanized due to urinary tract obstructions; as a result, this may have limited the use of results from our study to fully represent the population of all goats with calcium carbonate uroliths because of this form of bias.

Lastly, higher prevalence of calcium carbonate uroliths that were observed in goat breeds of African descent might have been due to the increase in the trend of keeping them as pets. As pets, they may have received more veterinary care when sick compared to goats kept for other purposes. This trend may have influenced the submission of uroliths from this group of goats compared to other breeds of goat that are used for other purposes.

Although information derived from extensive urolith data compiled by the Minnesota Urolith Center provides a unique resource for retrospective study, but because we have limited access to goats with uroliths, we have directed our efforts toward the evaluation of the epidemiological features of this disorder. Knowledge of the epidemiology of urolithiasis is essential for constructing effective experimental designs and choosing appropriate cases and controls for conducting clinical trials with meaningful results that may facilitate development of surveillance strategies that result in earlier detection of uroliths in goats.

Table 1—Logistic regression analysis results indicating crude and adjusted ORs and 95% CIs for breed and breed origin of case goats with calcium carbonate uroliths (n = 354) and control goats (16,366).

Breed	**No. of animals***		**Crude OR**	**95% CI**	**Adjusted OR†**	**95% CI**	***P* value**
	Cases	**Controls**					
Pygmy	105	2,715	1.0	NA	NA	NA	Referent
Boer	31	794	1.009	0.67–1.51	1.002	0.59–1.49	0.986
Alpine	24	1,415	0.44	0.28–0.69	0.325	0.27–0.65	0.003
Saanen	31	997	0.80	0.54–1.21	0.76	0.51–1.11	0.294
Angora	10	440	0.59	0.30–1.13	0.54	0.27–1.10	0.112
La Mancha	6	655	0.24	0.103–0.542	0.21	0.100–0.48	0.006
Mixed	10	4,214	0.061	0.032–0.12	0.051	0.022–0.110	< 0.001
Nigerian Dwarf	10	22	11.75	5.43–25.45	10.98	5.42–24.68	< 0.001
Anglo-Nubian or Nubian	25	3,900	0.17	0.11–0.26	0.15	0.10–0.25	< 0.001
Oberhasli	3	120	0.65	0.203–2.08	0.63	0.20–2.04	0.468
Toggenburg	7	970	0.187	0.087–0.402	0.17	0.073–0.38	< 0.001
Goats of non-African origin	129	12,803	1.0	NA	NA	NA	Referent
Goats of African origin	146	3,439	4.21	3.32–5.36	3.85	3.10–4.77	< 0.001

Case animals were defined as goats for which the uroliths were submitted to the Minnesota Urolith Center[a] between January 1, 1984 and December 31, 2012 and were found to contain ≥ 70% calcium carbonate. Controls were goats without urinary tract disease evaluated at veterinary teaching hospitals during the study period (identified through use of the Veterinary Medical Database[b]).

*Breed-specific analysis excluded case goats (n = 13) of various breeds for which no controls were available; and case goats (n=79) or control goats (n=124) for which breed was not recorded. Breed origin analysis excluded 79 case and 124 control goats for which this variable was unknown. †Adjusted for age, sex, and reproductive status as confounding factors and their interactions. NA = Not applicable.

Table 2—Logistic regression analysis results indicating crude and adjusted ORs and 95% CIs of demographic variables for case goats with calcium carbonate uroliths (n = 354) and control goats (16,366).

Variable	Crude OR	95% CI	Adjusted OR†	95% CI	*P* value
Age					
< 1 year	1.0	NA	NA	NA	Referent
1 to < 2years	21.43	8.44–54.45	20.56	7.92–53.62	< 0.001
2 to < 4years	45.86	18.73–112.29	44.43	17.33–112.13	< 0.001
4 to < 7years	60.32	24.52–148.35	59.46	23.66–147.17	< 0.001
7 to < 10years	53.83	20.66–140.27	52.63	20.26–139.44	< 0.001
≥ 10years	23.81	7.22–78.44	22.6	7.12–77.48	< 0.001
Combined age category					
≥ 4 years	1.0	NA	NA	NA	Referent
< 4years	0.32	0.251–0.399	0.26	0.23–0.0.34	< 0.001
Sex					
Female	1.0	NA	NA	NA	Referent
Male	197.89	63.48–616.89	196.36	62.59–612.52	< 0.001
Reproductive status					
Sexually intact	1.0	NA	NA	NA	Referent
Neutered	57.13	40.66–80.28	56.48	39.43–78.95	< 0.001
Season					
Spring	1.0	NA	NA	NA	Referent
Winter	2.88	1.97–4.201	2.88	1.97–4.201	< 0.001
Summer	2.53	1.75–3.67	2.53	1.75–3.67	< 0.001
Fall	5.37	3.80–7.59	5.37	3.80–7.59	< 0.001
Geographic location					
Southwest	1.0	NA	NA	NA	Referent
Southeast	2.21	1.24–3.94	2.21	1.24–3.94	0.007
Midwest	1.11	0.64–1.92	1.11	0.64–1.92	0.070
West	12.27	7.19–20.95	12.27	7.19–20.95	< 0.001
Northeast	11.79	6.43–21.60	11.79	6.43–21.79	< 0.001
Breed origin and study period					
1984–1998					
Non-African breeds	1.0	NA	NA	NA	Referent
African breeds	6.09	3.12–11.97	5.53	2.87–10.66	< 0.001
1999–2012					
Non-African breeds	1.0	NA	NA	NA	Referent
African breeds	2.12	1.63–2.75	2.11	1.62–2.73	< 0.001

*Age-specific analysis excluded 58 case and 1,476 control goats for which age was not recorded. Sex and reproductive status analysis excluded 8 case and 197 control goats for which information was not provided. Breed origin analysis for the 2 study periods excluded 79 case and 124 control goats for which this variable was unknown.

See **Table 1** for remainder of key.

Table 3—Logistic regression analysis results indicating crude and adjusted ORs and 95% CIs for diet used in case goats with calcium carbonate uroliths (n = 354) and control goats (16,366).

Diet	**Case**	**Crude OR**	**95% CI**	**Adjusted OR†**	**95% CI**	***P* value**
Hay plus pelleted grain mix.	12	1.0	NA	NA	NA	Referent
Pasture	107	11.32	6.4-19.6	10.12	5.5-18.8	< 0.0001
Grass hay	74	7.92	4.62-13.54	7.02	3.7-13.16	< 0.0001
Alfalfa hay	37	4.56	1.89-6.93	3.51	1.8-6.85	0.0002
Commercial feed	55	6.28	2.8-10.03	5.22	2.7-9.92	< 0.0001
Whole grain	26	3.52	1.3-4.99	2.47	1.2-4.94	0.0116
Unknown	43					
Total	**354**					

Figure 1—Photograph of calcium carbonate uroliths (100% $CaCO_3$) from a 4.6-year-old neutered male goat. Measurements are shown in centimeters.

CHAPTER 5

Epidemiological evaluation of the risk factors for silica uroliths in goats (106 cases): Perspectives from the Minnesota Urolith Center.

SUMMARY

The study reported here was a retrospective case-control study to identify risk factors for silica urolith formation in goats. It included 106 goats with silica and 16,366 goats without urinary tract diseases. The Mean ± SD age of goats with silica urolith was 3.1 ± 1.7 years and range (2 months to 10 years). Pygmy and Nigerian Dwarf breeds of goat had higher odds of developing silica uroliths than mixed breed (reference group). Breeds of African origin (Pygmy, Nigerian Dwarf and Boer), comprised 49 of 90 (54 %) goats with silica uroliths with data reported. Goats of African descent had higher risk of silica uroliths than goats of non-African descent (reference group).

Male goats had higher risk for silica uroliths than female goats. Neutered goats had higher odds for silica uroliths detection, compared to sexually intact. Age category, geographic location, and season were also associated with detection of silica uroliths in goats. The proportion of goat silica urolith submissions to the MUC had decreased from 20% between1984 and1998 to 11% between1999 and 2012. The mean age of goats with silica uroliths increased from 2.3 ± 3.2 year between 1984 and1998 to 3 ± 2.5 between 1999 and 2012.

Males comprised 96% of goats with silica while females comprised only 4% between 1984 and 1998. Between 1999 and 2012 males comprised 100% of all goats with silica uroliths submitted to the MUC. There was no urolith samples retrieved from females and submitted to the MUC during this later study period. Results suggested that the prototypical goat with silica uroliths was a neutered male, 2 to < 7 years of age and of African descent. Our study identified risk factors associated with silica urolithiasis in

goats; however, these associations do not allow conclusions regarding cause-and-effect relationships. Identification of risk factors may be useful in the development of recommendation strategies for effective medical management of this condition.

INTRODUCTION

The name silicon (Si) is derived from Latin word "silicis" meaning flint. Silicon is a naturally occurring nonmetallic element. The term silicate is a noun used to designate a salt derived from silicic acid (as in aluminium silicate and magnesium trisilicate). This term should not be confused with silicone. Silicone includes any of a group of synthetic resins, oils, greases, plastics, etc, in which the carbon element has been replaced with silicon. Inorganic silica, whose formula is SiO_2, occurs naturally as crystalline, microcrystalline, cryptocrystalline or amorphous forms. [92-93]The designation SiO_2 as crystalline refers to the orientation of silicon molecules as a fixed, orderly and repetitive pattern resulting in a characteristic shape. Crystals reflect internal order of a substance. The term microcrystalline refers to the fact that crystals are so small that they cannot be seen through a microscope. The term cryptocrystalline refers to the fact that the crystals are too small to be seen by light microscopy. The designation as amorphous (without shape) refers to the orientation of SiO_2 molecules in a random or non-periodic pattern. When silicon combined with oxygen, silica or silicon dioxide is formed (SiO_2).[92-93]Silica is the most abundant mineral and constitutes more than 90% of the earth's crust. It occurs in low concentrations in most animals because of its low solubility in all but a few naturally occurring waters.[92-93] In comparison with animals, plants often contain higher quantities of silica. For instance, grass may contain 1-4% of silica by dry weight, while horsetail (a type of plant Equisetum hyemale) may contain up to 16% of silica by dry weight.[94] Silica uroliths are among the least commonly reported types of urolith in animals. Apparently, the first report of naturally occurring silica in animals did not appear in English literature until 1976 in a 4-year-old male German shepherd dog. [95] One plausible explanation as to why canine silica uroliths began to be recognized in the mid-1970s was that at that approximate time, the pet food industry started use of an increased quantity of plant-derived ingredients in moist and particularly dry dog foods. Uroliths containing 70% or greater silica accounted for 93,214 of 818,284(0.39%) of all canine uroliths submitted to the MUC and 293 of 71,049 (0.41%) of all uroliths submitted in 2015 (Unpublished report).

Between 1981 and 2007; a total of 941 uroliths retrieved from 19 ruminants were sent to the MUC [a] for analysis. The prevalence of silica was highest in Camels, Llamas, cows, and sheep. During this time period, 15 % of all goat uroliths submitted to the MUC were composed of silica.[8] Silica uroliths have been reported to be common in range herbivores, like sheep and cattle, that consumed foraged grasses containing a high concentration of silica. [96-97] Species differences exist in the prevalence rate of silica urolithiasis as it has been reported to be 0.2% in humans. [98] These rare cases of silica urolithiasis that have been reported in humans were not associated with ingestion of foods containing silica, but rather were associated with consumption of large quantities of antacids containing magnesium trisilicate used to alleviate signs of peptic ulcers.[99] Zonisamide ®, an antiepileptic drug, has been implicated in some human cases of silica urolithiasis. [99] In general, silica uroliths retrieved from man and animals appear to come in two forms: oval and jackstone configurations. The jackstone configuration was used because their shapes or configurations resemble that of a small six-pronged metal pieces used in children's game of jacks.[92]

According to a study reported in 2014, the United States was identified as the leading silica sand supplier in the world by providing materials that are essential for modern living.[100] Mineral exploration is an activity that is fast growing in the United States of America. This activity is believed to be responsible for the increase in the spread of silica dusts onto plant surfaces and soil by way of airborne routes in these regions. Furthermore, exposures of some plants to silica rich soil due to volcanic activities in some regions of the USA may have contributed to the increased levels of silica sand in water and plants that are found in the affected areas. [101, 102] Subsequently, it has been observed that silica uroliths are common in plant materials that are grown on soil contaminated with silicone dioxide which is a building block for silica uroliths formation.[101, 102] The formation of silica uroliths in goats is not fully understood, but some investigators have suggested that when plants contaminated with silica are consumed, silicate is converted to silicic acid in the rumen of the affected animals, thereby setting the stage for the formation of silica urolithiasis. After absorption by the kidney, silicic acid is converted to siloxane ($C_4H_{14}OSi_2$) which is then deposited in the urinary tract of the affected animals to form silica uroliths.[101, 102]

Furthermore, the association between food and formation of urolithiasis in goats is relevant to consideration of diet–related risk factor in goats. Urolithiasis has been reported in horses, sheep, cattle, dog and rat by other investigators.[10, 103- 105]While a lot of studies have been performed on urolithiasis of other ruminants, horses, dogs and cats, not much is known about urolithiasis in goats.

The purpose of the study reported here was to (1) Investigate epidemiological data on silica urolithiasis in goats. (2) Assist veterinarians in the surveillance, detection and prevention of silica urolithiasis in goats. (3) Prevent unnecessary euthanasia of goats because of this disease. (4) Determine if breed, age, sex, reproductive status, anatomic location within the urinary tract, season of uroliths collection, and geographic location of uroliths were risk factors associated with silica urolith formation in goats. (5) Determine if the rate of silica uroliths retrieved from goats and submitted to MUC varied over time. We hypothesized that breed, age, sex, reproductive status, geographic location, and season of urolith submission were risk factors associated with silica urolithiasis in goats.

MATERIALS AND METHODS

Identification of cases and controls and medical records review— Medical records of the MUC[a] were reviewed to identify goats for which urolith samples were submitted by veterinarians within the United States between January 1, 1984 and December 31, 2012. The mineral composition of the uroliths was determined by optical crystallography [42-43] and, when necessary, by infrared spectroscopy.[43] Case animals were identified as goats that had uroliths consisting of ≥ 70% silica. Animals with compound uroliths (i.e., those containing nuclei, stone, shell, or surface crystal layers with ≥ 2 layers with < 70% different mineral types) were excluded.

Control sample consisted of goats without urinary tract disorders evaluated at the veterinary teaching hospitals in the United States within the same period and were identified by searching the records of the Veterinary Medical Data Base (VMDB)[b]. Only animals that had urinalysis performed and had no urinary tract disease were selected as controls. Preliminary evaluation of case series data indicated that uroliths were not detected in goats < 2 months of age, so goats of this age in both cases and controls group were censored.

The following information was retrieved from uroliths submission records of each case animal and medical records of controls when applicable: breed, age, sex, reproductive status (neutered or sexually intact), state of residence, date of urolith submission, anatomic location of the urolith upper urinary tract [kidneys and ureters], lower urinary tract [bladder, urethra, and voided samples], upper and lower urinary tract (when retrieved from both locations) or unknown (when the retrieval location was not recorded in the submission forms), and diet (first and second components listed on the record). Distinction between the Anglo-Nubian and Nubian goat breeds could not be consistently determined from medical records of goats with uroliths submitted to the MUC. Therefore, goats of these 2 breeds were grouped together in the present study. Geographic regions[j] (Southeast, Southwest, Midwest, Northeast, or West) were determined on the basis of state of residence according to classification established by the National Geographic education division.[c] Seasons[i] of sample collection were classified as winter (December, January, February), spring (March, April, May), summer (June, July, August), and fall (September, October, and November).

Statistical analysis- Standard statistical software [44, e f g & k] was used to determine descriptive statistics (mean, median, and SDs) of breed, age, sex, reproductive status, and geographic location of goats; season of urolith submission and anatomic location of the uroliths within the urinary tract for case animals. Logistics regression was used and ORs and 95% CIs were calculated by use of Woolf method [45] to assess whether breed, age, sex, reproductive status, season and geographic location were associated with silica urolithiasis in goats. If any expected cell frequency in a contingency table was < 5, the

Fisher exact test was used.[46] Breeds were further categorized as Africa (Pygmy, Nigerian Dwarf, and Boer) or non-African (all others) in origin. For analysis of age effect, goats were divided into 6 age groups: < 1 year, 1 to < 2 years, 2 to < 4 years, 4 to < 7 years, 7 to < 10 years and ≥ 10 years. Data for goats < 4 years of age were also compared with those for goats' ≥ 4 years old. Referents for variables (determined on the basis of breaks in the data and mean age) for determination of ORs were as follows: breed, mixed; breed origin, non-African; age, < 1year; sex, female; reproductive status, sexually intact and intact female; season, summer; geographic region, Southwest; and changes over time, 1984-1998.

Prevalence of silica uroliths was expressed as relative frequencies of all the uroliths submitted for analysis to MUC. Relative frequencies also were used to describe the breed, age, sex, reproductive status, and geographic location of goats, as well as season of collection, and anatomic location of uroliths. This computation was performed to determine whether the percentage of uroliths retrieved from goats with silica submitted to the MUC increased or decreased over the study period. The 28-year study was grouped into 2 intervals ([1984 through 1998], and [1999 through 2012]) to determine whether risk or protective factors changed over time. The Breslow-Day statistic was computed to determine whether ORs were homogenous over the 2-time intervals.[47] The Mentel-Haenszel summary of OR was computed when the results of the Breslow-Day test were not significant. Values of $p < 0.05$ were considered as significant.

Because of the absence of continuous variables, OR values were computed using hierarchical well-formulated modeling to find the best risk model for the categorical variables of breed, age, sex, reproductive status, geographic location, season and anatomic location. After adjusting for age, sex and reproductive status as confounding factors and interactions, risk factors associated with urolith formation 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 [48]. On the basis of recommendations by Lilienfeld and Stolley [49], we classified significant ORs between 1.1 and 1.9 and ORs between 0.5 and 0.9 as weak associations. We interpreted significant ORs > 2 (i.e., risk) and ORs < 0.5 (i.e., protective) as clinically (biologically) important. All analyses were performed with standard software. [44, c &d] Results were considered as significant at values of $P < 0.05$ at 95% CI.

RESULTS

Mineral composition of uroliths– Uroliths retrieved from 832 goats was submitted to the MUC for analyses during the study period. Of these, uroliths containing ≥ 70% of silica comprised (n=109[13%]) of the submissions (**Table 1 & Figure 1**). After censoring submissions from outside the United States (n=3), silica content of the remaining 106 samples from 106 case goats was as follows: 100%, 84 (79%) samples; 90% to 99%, 17 (16%); and ≥ 70% to 89%, 5 (5%). Silica uroliths retrieved from goats were typically spherical and smooth (**Figure 2**). They were cream or ash color and range from < 1 mm to > 1 cm in diameter. The number of uroliths submitted from each case goat varied.

Breed- Case goats included 15 breeds and control goats included 11 breeds. Four breeds were excluded from breed-related analysis because of lack of controls animals, these included Fainting (n=2 case goats), Cashmere (1), Kinder (1) and Pygora (n=2). Breed data for 16 of 106 (15%) case animals and 124 of 16,366 (0.8%) control animals were not provided at the time of urolith submission. The breeds with the higher odds of developing silica uroliths, compared to mixed breed, were Alpine, Anglo-Nubian, Angora, Boer, La Mancha, Pygmy, Nigerian Dwarf, Oberhasli, Saanen and Toggenburg. None of the breeds in this study had lower odds of developing silica urolith, compared to mixed breed. Of the 90 submissions where breed was recorded, 49 of 90 (54%) were of African origin and 41 of (46%) were non-African origin. Goats of African descent had significantly (**Table 2 & 3; P< 0.001**) higher odds of developing silica uroliths than the referent group (OR, 2.7). From 1984 through 1998, case goats of African descent constituted 8 of 20 (40%) goats with silica uroliths. Goats of non-African descent constituted 12 of 20 (60%) of this group. During the same period, 1,579 of 11,205 (14%) control goats were of African descent, and the remaining 9,626 (86%) were breeds with other origins. From 1999 through 2012, 40 of 70 (57 %) and 30 of 70 (43 %) case goats were of African and non-African descent, respectively. Among 5, 037 controls goats seen during this period, breeds of 1,816 (36%) and 3,221 (64%) were of African and non-African origin respectively. Breed origin data were unavailable for 16 case and 124 control goats seen during 1984 through 2012 (**Tables 2 & 3**).

Age- Mean ± SD age of 94 case goats with silica uroliths for which data were available was 3.1± 1.7 years and range (2 months to 10 years). Sixty of 94 (64%) of these case goats were < 4 years and 34 of 94 (36%) were ≥ 4 years of age. The odds of developing silica uroliths were significantly greater for goats of 2 to < 7 years, compared to the odds for goats of < 1 year of age (Referent group [**Tables 3]**). When combined age groups were evaluated, goats that were < 4 years old were less likely (adjusted OR, 0.40) to develop silica uroliths compared to goats of ≥ 4 years old (reference group [**Table 3**]).

Gender and Reproductive Status - Of 106 case goats, sex was recorded for 103 (97%). Of the 103, 101 of 103 (98%) were males and 2 of 103 (1.9%) were females. Males were approximately 86 times as likely to develop silica uroliths as were females (referent group; table 2). The reproductive status for 103 of 106 (97%) case goats was recorded; 87 of 103 (84%) of these were neutered and 16 of 103 (16%) were sexually intact. A higher proportion of neutered male goats (87 of 103[84%]) had silica uroliths, compared to sexually intact males (16 of 103[16%]; $P < 0.001$). Neutered goats were approximately 37 times as likely to be detected with silica uroliths compared to sexually intact goats (**Table 3**).

Anatomic location- Sites from which the uroliths were collected were reported for 102 goats and unknown for 4. Ninety-nine of 102 (97%) goats had samples obtained from the lower urinary tract (urinary bladder, urethra, voided uroliths or some combination of these). Samples from 2 of 102 (2%) were retrieved from upper urinary tract (Kidneys and ureter). Submissions from both upper and lower urinary tracts were 1 of 102 (1%). Submitted uroliths were from the bladder only for 25 of 102 (25%) goats, and from the urethra only for 56 of 102 (55%) goats. Submission of uroliths retrieved from the kidney (n=2) or ureter/bladder (n=1) was uncommon (**Table 5).**

Seasonal distribution- Submission of uroliths for the 106 case goats took place in fall (n=22 [21%], summer (12 [11%]), winter (42 [40%]) and spring (30[28%]). Silica uroliths were significantly (OR= 3.5; $P < 0.001$; winter) more likely to be collected and submitted in winter, than in summer [Referent group; (**Tables 3**)].

Geographic distribution –Four of 106 (3.8%) case goats were received from the Southwest, 10 of 106 (9%) were received from the Southeast, 21 of106 (20%) were received from the Midwest, 52 of 106 (49%) were received from the West and 19 of 106 (18%) were received from the Northeast geographic zones. The frequency of submission of silica was approximately 20 times greater in the Northeast compared to Southwest (referent group; [Table, $P< 0.001$]). Likewise, the frequency of submission of silica uroliths in the West were approximately 14 times greater in the West compared to Southwest ([Referent group; [**Tables 3; P= < 0.001**]).

Diet-Of the 106 goats with silica uroliths, the primary type of diet was recorded for 72 of 106 (68%). Of these, 50 % (n=36) were fed grass/pasture, 22% (n=16) were fed grass hay, 13% (n=9) were fed alfalfa hay, 5% (n=4) were fed commercial food type (type not specified in the submission forms), 7% (n=5) were fed whole grains, corn, wheat etc., and 3% (n=2) were fed pelleted grain mixture (pelleted grains blended with vitamins and minerals). In 32% (n= 34) of goats the types of diet used were not recorded in the submission forms.

Silica urolith and other minerals changes over time-The proportion of silica urolith submissions to the MUC in relation to other minerals decreased from 26 of 133 (20%) between 1984 and 1998 to 80 of 699 (11%) between 1999 and 2012. On the other hand, the non-silica minerals (other minerals save silica) increased in relation to silica from 107 of 133 (80%) between 1984 and 1998 to 619 of 699 (89%) between 1999 and 2012 (**Table 6**). From 1984-1998, the Mean ± SD age of goats with silica was 2.3 ± 3.2 years, and males constituted 96% while the females constituted 4%. From 1999-2012 the Mean ± SD age of goats with silica increased to 6 ± 2.5 years, and males constituted 100% of the submissions during that latter period. None of the silica urolith submissions during the second part of the study (1999-2012) was from a female goat (**Tables 3**).

DISCUSSION

Although silica comprises more than 90% of the earth's crust, silica uroliths still occur with low frequency in most domestic animals (i.e. cats and dogs).[24] Results of our study support the hypothesis that multiple risk factors such as breed, diet, age, sex, reproductive status, geographic location and season of urolith submission play a role in the formation of silica uroliths. Similar results had been made by other investigators.[105-108] However, those studies,[102-105] were performed without controls. Instead generalities they observed were based on uncontrolled empirical observations.

As theorized in urolith formation in dogs and cats, it is probable that when several risk factors occur together, their individual effect may be enhanced. Thus each risk factor or protective factor may play either a limited or a major role in the development or prevention of silica uroliths.[77] However, case-control studies usually cannot demonstrate that risk factors are the cause of a disease. The case animals in the present study were likely goats with clinical signs and may not have included subclinical cases, such as goats that voided uroliths uneventfully, or those whose uroliths were not collected and submitted to the MUC for various reasons (e.g. goats that died as a result of urinary tract obstruction or goats that were euthanized because of complications associated with urinary tract obstructions). As a result of these limitations, this study may not have fully represented the population of all goats with silica uroliths.

The results of our study indicated that goat breeds of African origin were at increased risk for silica urolithiasis detection, compared to other breeds (i.e., those of non-African origin). Goat breeds of African descent (Pygmy, Nigerian Dwarf, and Boer) were likely overrepresented among case goats with silica uroliths (i.e., case animals) in our study. This higher prevalence of silica uroliths in goat breeds of African descent may be associated with a trend of keeping them as pets. As pets, they may be more likely to receive veterinary care when sick, compared to animals kept for other purposes. The number of goats with silica uroliths in this study may have reflected an increased awareness that uroliths can cause urethral obstruction and increased use of diagnostic methods to detect uroliths such as survey radiography and abdominal ultrasonography in the latter half of the study period. This may also be associated with an increased awareness of the availability of complimentary urolith analysis in MUC that may have influenced submissions made during these 2 periods ([1984 through 1998] and [1999 through 2012]) [a]

The result of our study indicated that goat breeds of African origin were at increased risk for silica urolithiasis, compared to other breeds (i.e., those of non-African origin). Goat breeds of African origin have been observed to routinely consume less amounts of water in their native continent. This pattern of water drinking usually stems from lack of water which is scarce in their native continent which in turn results in the formation of concentrated urine.[106-108]It is a common knowledge that concentrated urine is a risk factor for urolith formation. Based on this knowledge the perception of less water consumption habit observed in goats of African origin may have been responsible for the increased odds for urolith formation seen in them. The adage which states that form good habits, they are hard to break as much as bad habits is true under such circumstance. It is much more difficult for goats of African origin to break these cultivated habits of less water consumption already developed in their native continent due to scarcity, even when they reside in regions with abundant water supply. Also the effects of cold weather on water consumption as observed during winter season in general is likely to have a more marked effects on goats of African origin compared to those of non-Africa origin. This observation of less water consumption may have resulted in the production of more concentrated urine and more tendencies to development silica urolithiasis compared to other breeds.   Familial predisposition for urolithiasis has been well documented for some mineral types.[69-72] For instance; genetic mutations causing cystine urolithiasis have been identified in dogs [69-72] and humans.[69-72] To the knowledge of the authors, a genetic predisposition for silica urolithiasis in goats has not been investigated. In our study, the observation that silica uroliths more commonly affected goats of African descent, compared to other breeds, may suggest genetic predisposition of these breeds to silica urolithiasis. Prospective studies evaluating the effects of diet, housing, body condition score, and other potential risk and protective factors in addition to breed genetic make-up are necessary to further investigate a potential predisposition to this type of urolithiasis.

In our study, age was significantly associated with development of silica urolithiasis in goats, with goats within the age groups of 2 to < 7 years having significantly higher odds for this finding, compared to goats of < 1year of age category (reference group). From 1984-1998, the Mean ± SD age of goats with silica was 2.3 ± 3.2 years, and from 1999-2012, the Mean ± SD age of goats with silica increased to 6 ± 2.5 years. Epidemiological data derived from studies.[13 -14] of dogs, cats and humans have suggested that advancing age is a risk factor for urolithiasis.

Our results also indicated that silica uroliths almost exclusively affected males (101/103[98%], compared to females 2/103[1.9]. From 1984-1998 silica urolith submissions retrieved from male and female goats and submitted to the MUC comprised 96% and 4% respectively of the total urolith submissions during that period. From 1999-2012 males constituted 100% of goats with silica urolith submitted to the MUC and none was retrieved from females and submitted to the MUC during the same period. Silica urolithiasis in goat is predominantly observed in males compared to females, since the females comprised only < 2 % of cases in this study. This is likely related to the fact that male urethras are substantially longer and narrower than those of females.[82] Also presence of urethral recess and urethra process seen in males only may have contributed to more severe clinical manifestations observed in males. As urocystoliths pass into the distal end of the male substantially longer and narrower urethra as well as the urethral process; obstructive urolithiasis is a likely consequence. The S-shaped sigmoid flexure located just caudodorsal to the testes of males is another potential site for uroliths to lodge. It is likely that silica uroliths are not commonly detected in female goats because the substantially wider and shorter urethra in females allows uroliths to be passed in the urine stream before they can cause clinical signs. A higher proportion of case goats 87/103[84%] were neutered more than were sexually intact 16/103[16%] and odds of this finding were significantly greater for neutered goats than for sexually intact goats (Table 3, OR, 37; P= < 0.001). Given that none of the 2 female case animals in this study was neutered, this statement may most appropriately be applied to neutered males only. This observation suggests that neutering may be a risk factor for urolithiasis. One group of investigators have generated convincing evidence that early neutering may result in underdevelopment of the urethra and thus predispose adult goats to urethral obstruction.[39] The same group of investigators have shown that neutering reduces production of testosterone which normally aids in the development of urethral lumen.[83] A reduced circulating testosterone concentration is also believed to diminish normal preputial-penile attachment.[83] Because the ages at which case goats in this study were neutered was not provided, we were unable to determine whether there was a significant association between silica urolithiasis and early neutering.

Ninety-nine of 102 (97%) case animals in this study had their silica uroliths retrieved from the lower portions of the urinary tract. The predominant location of uroliths in the lower portions of the urinary tract in other domestic animals has been previously described.[84]In our experience in other species, the development of uroliths in the upper portions of the urinary tract is likely underreported, because survey radiography and ultrasonography are often omitted during initial evaluation of animals with clinical signs of urolithiasis. Clinical signs caused by uroliths in the lower portions of the urinary tract (increased voiding frequency, staining to void, and dribbling urine) are more likely to be observed than are clinical signs caused by uroliths located in the upper urinary tract (polyuria and hematuria without obvious signs of pain, which may go unrecognized). In the case animals of the present report, uroliths were commonly retrieved from the urethra only (56/102[55%] more than that retrieved from the bladder only (25/102[25%]. These localizations are important when considering dietary modification in dissolution of uroliths. It has been recommended that attempts to dissolve urethral uroliths by dietary modification be avoided. [84] Uroliths lodged in the ureters or urethras cannot be dissolved by medical protocols because urolith dissolution requires sustained contact with urine that has been modified and therefore is undersaturated with lithogenic minerals.[84]

In our study, goats residing in the Northeast and Western regions had significantly higher odds of developing silica urolithiasis than did goats from the other regions of the United States. It has also been shown that grasses contain between 1 and 4 % of silica per dry weight, while rice hulls and scouring rushes such as horsetails and Equisetum can contain up to 16% of silica per dry weight in certain geographic regions of the United States. Prairie grass such as Festuca scabrella that is commonly seen in Canada has been found to contain between 4 to 8% of silica per dry weight.[103] Also it has been shown that silica sand excavation is a risk factor for silica urolithiasis and is a common activity in the above regions.[109] These observations indicate that geographic location may be risk factor for urolithiasis. Our results are in agreement with other investigators who have reported the association between silica soil content, volcanic activities and silica urolithiasis. [101-102, 109] Geographic prevalence of urolihiasis has been reported in dogs and humans.[85-86] Our study was not designed to determine the reason for the variations among geographic locations. Further research is needed to assess the factors that contribute to such differences.

Silica uroliths were significantly (**Table 3; P= < 0.001**) more likely to be retrieved from goats and submitted to the MUC during winter than during other seasons. The association between season and prevalence of urolithiasis observed in this study is in agreement with findings by other investigators, who reported that detection of urolithiasis in cats, sheep, and humans varies with seasons; with odds in winter being 3 times more than what is observed in summer (**Table 3; P= < 0.001**).[87] This may be partly attributable to reduced water intake during winter season which could lead to increased urine concentration and subsequently increases the risk for silica urolithiasis. Similarly, higher seasonal temperature that is observed in fall has also been shown to lead to heat-induced water loss in comparison to winter and spring which can also cause concentrated urine and subsequently may lead to uolithiasis.[87] We did not investigate possible association between silica urolithiasis and these factors. Our study was not designed to explore the reason for urolith formation in the face of temperature variations.

Diet has been incriminated as important risks factor in silica uroliths formation in goats.[90] In our study; we encountered a lack of detailed information regarding diet composition and variability in the reported diets fed to case goats. Lack of consistency in response to our requests for dietary history prevented evaluation of dietary components as risk factors for this disease. To our knowledge, controlled, blinded clinical studies have not been performed in goats to evaluate associations between most dietary risk factors and urolith formation in goats. Our study was also not designed to explore this factor, and we were unable to test for such associations, although it is probable that diet has a role in the pathogenesis of silica urolithiasis. In future, prospective study will be used to determine the role which diets play in the pathogenesis of silica uroliths formation.

However, most of the case goats for which diet was reported primarily received alfalfa hay and grass hay which comprised the bulk of the feeds recorded on the completed submission forms (50%). Alfalfa and grass hay contain a high calcium/magnesium ratio and low phosphorus. Alfalfa and grass hay are also rich in oxalate. The fact that ingested silica is rapidly cleared by the kidneys from plasma of dogs and other animals following intestinal absorption into the body also supports the “dietary risk factor” hypothesis.[110-112] Monocotyledonous plants are observed to be common in the Northeast, and Western regions of the United States. Most of the plants fed to the goats in our study were composed of grass hay, and alfalfa hay which are monocotyledonous and are known to be rich in silica. This observation is in agreement with the results of our study which observed that silica urolithiasis is more common in the Northeast and Western regions of the United States of America. Furthermore, prospective studies are needed to define the role played by each of these factors singly and in combination as they relate to silica uroliths formation.

Healthy goats normally excrete urine that is alkaline (pH range, 7.5 to 8.5). Sheep, rabbits and horses also normally excrete alkaline urine.[91] Production of alkaline urine has been cited as a potent risk factor in these herbivorous species. Most of the plants used to feed goats in our study (e.g., grass, grains, and alfalfa) are associated with alkaline urine production.[91]Although, not investigated in our study; it is probable that alkaline urine pH is a risk factor for silica uroliths formation in goats.

The results of our study indicated that the proportion of silica urolith submissions to the MUC in relation to other lithogenic minerals decreased from 26 of 133 (20%) between 1984 and 1998 to 80 of 699 (11%) between 1999 and 2012. However, other minerals save silica increased from 107 of 133 (80%) between 1984 and 1998 to 619 of 699 (89%) between 1999 and 2012. Our results are in agreement with current epidemiological reports which have indicated that silica uroliths remained uncommon in most places.[113]One exception to this generality was a report on dogs in Mexico City in which the reported proportion of silica uroliths increased by 14%. [113] The high concentration of silica observed in drinking water in the said region (Mexico City) were likely responsible for the increased incidence.   However, since the Minnesota Urolith Center comparatively received from very few to zero urolith samples for analysis from South America, our data may not have provided a suitable comparison for the distribution of silica uroliths affecting goats in that geographic location. So we take this information as stated without having to compare it to our results because of the above stated reason.

The possible links between silica urolithiasis in goats and soil silica content has generated a lot of interests within the veterinary clinical research community. Although available clinical data have provided a strong link between silica uroliths and dietary ingredient, but knowledge of the part played by each ingredient in the feed is still unknown. Based on the biological behavior of silica uroliths in other species it is believed that it tends to recur following surgical removal. Therefore, identification of the risk factors associated with silica urolithiasis in goats will help in the prevention of recurrences that may occur in this condition. Although information derived from extensive urolith data base compiled by the MUC provides a unique resource for retrospective case- control study on urolithiasis, but because we have limited access to goats with uroliths, we have directed most of our efforts toward the evaluation of the epidemiological features of this disorder. Knowledge of the predominant mineral types of uroliths in goats, 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 goats. Modification of risk factors, where possible, may help to minimize urolith formation, dissolve existing uroliths and minimize recurrences. Also this knowledge will be essential for constructing effective experimental designs and choosing appropriate cases and controls for conducting clinical trials with meaningful results that may facilitate development of surveillance strategies that may result in earlier detection of silica urolithiasis in goats.

Biases and limitations. Since our data was obtained from goat's silica urolith submissions to the MUC, it was not a complete representative of all urolith submissions from all breed, age, sex, reproductive status, season and geographic location of all goat populations. Urethras of male goats are substantially longer and narrower than females. Also the males have urethral process and sigmoid flexure which are absent in females. All these features may have contributed to more clinical manifestations of urethral obstruction in males compared to females and may have therefore influenced their selection as silica urolith cases. Since the MUC uroliths analyses are performed at no cost to veterinarians this may have increased the rate of urolith submissions to the center. Because the sample size in this study (n=106) was not very large we were unable to apply the conclusions derived from the findings of this study to the whole goat population with silica urolithiasis.

Silica uroliths obtained from goats in this study were retrieved mainly from the lower urinary tract. Clinical signs are more likely to be observed when uroliths are located in the lower urinary tract than from the upper urinary tract. This may have constituted a form of bias in this study leading to a higher number of lower urinary tract silica being observed in this study compared to upper urinary tract silica. Furthermore, this study may have included more clinical cases, and may not have included asymptomatic cases or cases that may have been euthanized because of urinary tract obstructions; as a result, this may have limited the use of results from our study to fully represent the population of all goats with silica uroliths. To date we have not been able to design a dietary protocol which will dissolve or prevent silica uroliths in animals and this will constitute the future direction of this study. Information obtained from these biases will be helpful in achieving this goal.

Table 1—Mineral composition[a] of 832 goat uroliths from USA (Capra hircus; domestic goat) evaluated at the MUC from 1984 to 2012

Mineral composition [a]	Number	(%) of uroliths
Calcium carbonate	364	44
Magnesium calcium phosphate carbonate	173	21
Silica	109	13
Magnesium ammonium phosphate hexahydrate	29	4
Magnesium calcium phosphate apatite	41	5
Calcium oxalate monohydrate	1	< 1
Calcium oxalate dihydrate	6	< 1
Calcium phosphate carbonate	5	<1
Calcium phosphate apatite	13	2
Xanthine	1	< 1
Mixed [b]	33	4
Compound [c]	46	6
Other	7	<1
Matrix	4	<1
TOTAL	**832**	**100**

[a] Analyzed by polarizing light microscopy or infrared spectroscopy.
[b] Uroliths did not contain at least 70% of one mineral type in the stone layer.
[c] Uroliths contained at least 70% of one mineral type in one layer and at least 70% of a different mineral type in a surrounding layer(s).

Table 2—Logistic regression analysis results indicating crude and adjusted ORs and 95% CIs for breed and breed origin of case goats with silica uroliths (n = 106) and control goats (n = 16,266).

Breed	**Crude OR**	**95% CI**	**Adjusted OR†**	**95% CI**	***P* value**
Mixed	1.0	NA	NA	NA	Referent
Alpine	14.89	1.74-127.57	13.49	1.65-127.66	0.0137
Anglo-Nubian	10.81	1.38-84.45	9.46	1.32-84.39	0.0233
Angora	28.73	2.98-276.81	26.66	2.67-276.94	0.0037
Boer	42.46	5.30-339.95	40.6	5.21-339.42	0.0004
La Mancha	25.73	2.87-230.61	23.64	2.69-230.13	0.0037
Nigerian Dwarf	80.93	34.86-276.06	78.43	33.78-226.05	< 0.001
Oberhasli	35.12	2.183-564.79	33.03	2.09-564.51	0.0120
Pygmy	51.22	7.001-374.72	49.12	6.81-374.55	< 0.001
Saanen	33.813	4.22-270.67	31.15	4.13-269.15	0.009
Toggenburg	13.03	1.35-125.43	10.96	1.27-125.45	0.0263
Goats of non-African origin	1.0	NA	NA	NA	Referent
Goats of African origin	4.45	2.93-6.75	2.69	2.86-6.67	< 0.001

Case animals were defined as goats for which the controls were submitted to the Minnesota Urolith Center[a] between January 1, 1984 and December 31, 2012 and were found to contain ≥ 70% calcium carbonate. Controls were goats without urinary tract disease evaluated at veterinary teaching hospitals during the study period (identified through use of the Veterinary Medical Database[b]).
Breed-specific analysis excluded case goats (n = 4) of various breeds for which no controls were available and case (79) or control goats (124) for which breed was not recorded. Breed origin analysis excluded 16 case and 124 control goats for which this variable was unknown. †Adjusted for age, sex, and reproductive status as confounding factors and their interactions.
NA = Not applicable

Table 3—Logistic regression analysis results indicating crude and adjusted ORs and 95% CIs of demographic variables for case goats with Silica uroliths (n = 106) and control goats (16,366).

Variable	Crude OR	95% CI	Adjusted OR†	95% CI	*P* value
Age					
< 1 year	1.0	NA	NA	NA	Referent
1 to < 2years	1.83	0.823-4.084	1.74	0.743-4.014	0.1388
2 to < 4years	4.42	2.411-8.109	3.33	2.311-8.019	< 0.001
4 to < 7years	5.95	3.183-11.119	4.89	3.048-11.039	< 0.001
7 to < 10years	2.67	0.88-8.034	1.72	0.79-7.954	0.0832
≥ 10years	1.32	.0174-10.046	1.26	0.0132-10,037	0.7868
Unknown					
Combined age category					
≥ 4 years	1.0	NA	NA	NA	Referent
< 4years	0.444	0.291-0.6772	0.364	0.201-0.5872	0.0002
Unknown					
Sex					
Female	1.0	NA	NA	NA	Referent
Male	87.404	21.55-354.43	85.95	21.46-354.32	< 0.001
Unknown					
Reproductive status					
Sexually intact	1.0	NA	NA	NA	Referent
Neutered	38.33	22.44-65.45	37.25	22.35-65.36	< 0.001
Unknown					
Season					
Summer	1.0	NA	NA	NA	Referent
Winter	4.54	2.39-8.64	3.46	2.31-8.55	< 0.001
Spring	2.092	1.069-4.091	1.492	1.009-4.041	0.0310
Fall	2.35	1.163-4.762	1.65	1.103-4.702	0.0173
Geographic location					
Southwest	1.0	NA	NA	NA	Referent
Southeast	1.594	0.499-5.090	0.894	0.429-5.023	0.4313
Midwest	0.9323	0.319-2.719	0.6325	0.239-2.629	0.8979
West	15.54	5.61-43.068	14.45	5.46-42.778	< 0.001
Northeast	21.53	7.285-62.649	20.43	7.195-62.569	< 0.001

Table 4—Logistic regression analysis results indicating crude and adjusted ORs and 95% CIs of changes over time and breed origin and study period for case goats with Silica uroliths (n = 106) and control goats (16,366).

Variable	**Crude OR**	**95% CI**	**Adjusted OR†**	**95% CI**	***P* value**
Changes over time					
1984-1998	1.0	NA	NA	NA	NA
1999-2012	7.37	6.67-11.64	6.49	6.59-11.58	< 0.001
Breed origin and study period					
1984–1998					
Non-African breeds	1.0	NA	NA	NA	Referent
African breeds	4.064	1.66-9.96	3.497	1.58-9.76	0.0022
1999–2012					
Non-African breeds	1.0	NA	NA	NA	Referent
African breeds	2.36	1.488-3.8099	1.621	1.408-3.739	0.0004
Unknown					

*Age-specific analysis excluded 13 case and 1,476 control goats for which age was not recorded. Sex and reproductive status analysis excluded 4 case and 197 control goats for which information was not provided. Breed origin analysis for the 2 study periods excluded 16 case and 124 control goats for which this variable was unknown. *See* Table 1 for remainder of key.

Table 5— Anatomic locations of silica uroliths from 106 goats from USA

Anatomic location	No./%
Bladder	25 (24.5)
Bladder/urethra	12 (11.8)
Kidney	2 (1.9)
Ureter/Bladder	1 (0.9)
Urethra	56 (54.9)
Void	6 (5.9)
Unknown	4 (3.8)
TOTAL	**106 (100)**
Lower tract	99 (97.1)
Upper tract	2 (1.9)
Lower/Upper	1 (0.9)
Unknown	4 (3.9)
Total	**106(100)**

Table 6—Comparison of silica urolith and other minerals submissions from goats between 1984 and 2012 (n=832).

Time interval	Silica	Non-silica	Total
1984-1998	20% (n=26)	80% (n=107)	100% (n=133)
1999-2012	11% (n=80)	89% (n=619)	100% (n=699)
Grand Total	13% (n=106)	87% (n=726)	100% (n=823)

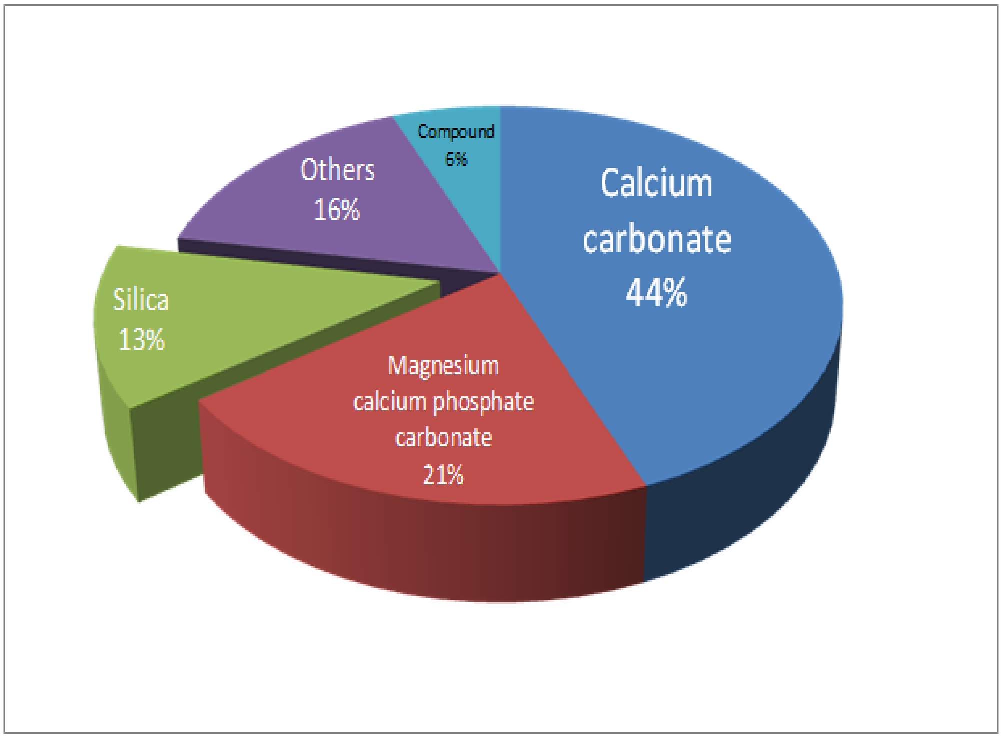

Figure 1— Prevalence of urolith types from goats: 1984-2012.

Others: CaOx, struvite, xanthine, magnesium calcium phosphate carbonate, magnesium phosphate apatite, calcium phosphate apatite etc.

Figure 2—100% silica uroliths from 2-year-old male castrate pygmy goat. Measured in centimeters

CHAPTER 6

Epidemiological evaluation of magnesium ammonium phosphate (a.k.a struvite) uroliths in goats.

SUMMARY

This retrospective case-control study evaluated urolith submission data of goats with struvite urolith. It involved 29 goats with struvite uroliths (case animals) and 16,366 goats without urinary tract diseases (control animals). It involved uroliths submitted to the MUC between January 1, 1984 and December 31, 2012. The Mean ± SD age of goats with struvite urolith was 1.8 ± 1.2 years and ranged from 3 months to 9.8 years. **S**truvite uroliths were common in Boer and Pygmy goat breeds. Breeds of African descent (Boer, pygmy and Nigerian Dwarf) comprised 14 of 20 (70%) of case goats with data available. Goats of African origin have higher risks for struvite uroliths formation compared to other breeds. Sexually intact goats comprised the bulk of goats with struvite uroliths (57%). More than 57% percent of goats with struvite uroliths were < 1 year of age. Geographic location and season were associated with detection of struvite uroliths in goats.

Goats with struvite uroliths were typically neutered males, < 1 year of age, and of African descent. This study identified rise factors associated with struvite urolithiasis in goats; however, these associations do not allow conclusions regarding cause-and-effect relationships.

INTRODUCTION

Goats are becoming increasingly popular as pets in the United States.[25-27] Leading this trend of popular pet goats are breeds of African descent.[25-27] The affectionate and docile nature of goats of African origin likely has contributed to their popularity as pets in the United States.[25-27] As of January 2014, the population of goats in the United States was estimated to be 2.3 million by the Agricultural Statistics Board.[75] Goats, along with being pets, are used for food, fiber, weed control, youth development organization projects, animal cell culture production, pack animal activities, and competition in livestock shows.[75] Not much is known about changes in diets and natural habits of these breeds of African goats in their native continent.[76]

As the population of pet goats increases, uroliths are being detected with increased frequency. One explanation may be that pet goats may receive more intensive medical attention than more traditional livestock. It is also possible that pet goats are more likely to be housed individually or kept at residences because of their small size and therefore are under closer observation than animals not kept as pets. Knowledge of the various mineral compositions of uroliths affecting goats and the frequency for urolith formation are needed to develop effective diagnostic, therapeutic, and preventive strategies.

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. [114] The name “struvite” was given to the mineral because the crystal is composed of magnesium, ammonium and phosphate hexahydrate (MgN_4HO_4). Prior to that time, struvite was sometimes referred to as "guanite" because the mineral was detected in bat guano.[114] 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. But this belief has since been debunked as not being true.

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 green due to coloration by bile pigment. They are usually opaque, round, elliptical, or faceted; some may grow in the shape of renal pelvis, ureters, bladder, or urethra. In cats and ferrets, struvite uroliths are usually pure and occur predominantly in the lower urinary tract.[60, 78] On the other hand struvite uroliths in dog and human are often not pure because they are often times associated with urinary tract infection.[57]

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.[60, 78] In cats and ferrets, risk factors associated with sterile uroliths include breed, age, sex, reproductive status, hydration status, urine pH, dietary composition, season of the year, anatomic location, and genetics. These risk factors may contribute to over-saturation of urine with magnesium, ammonium, and phosphate. To the authors' knowledge urinary tract infections with urease-producing microbes have not been reported to be of common occurrence in goats.

Struvite uroliths may be suspected in goats just as is presently done 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 urolith may play key role in the development of safe and effective non-surgical treatment methods for struvite urolithiasis.

The number of struvite uroliths retrieved from goats and analyzed at the MUC progressively increased from 3 in the period of 1984 to 1994, to 20 in the period 2002 to 2012. As the frequency of detection of goats' uroliths increases, knowledge of the different mineral types and associated risk and protective factors for uroliths formation is needed to develop effective diagnostic, management, and prevention strategies. The purpose of the study reported here was to determine the prevalence of naturally occurring struvite uroliths retrieved from goats, and submitted to the MUC; and determine whether breed, age, sex, reproductive status, anatomic location within the urinary tract, and season of detection and geographical location of urolith-formers were risk factors associated with struvite urolith formation.

MATERIALS AND METHODS

Identification of cases and controls and medical records review—Medical records of the Minnesota Urolith Center[a] were reviewed to identify goats for which urolith samples were submitted by veterinarians within the United States between January 1, 1984 and December 31, 2012. The mineral composition of the uroliths was determined by optical crystallography[42, 79] and, when necessary, by infrared spectroscopy.[42] Case animals were identified as goats that had uroliths consisting of ≥ 70% struvite. Goats with compound uroliths (i.e., those containing nuclei, stone, shell, or surface crystal layers with ≥ 2 layers with < 70% different mineral types) were excluded. Preliminary evaluation of case series data indicated that uroliths were not detected in goats < 3 months of age, so goats of this age in both cases and controls group were censored.

Controls are goats without urinary tract disorders evaluated at the veterinary teaching hospitals in the United States between January 1, 1984, and December 31, 2012, and were identified by searching the records of the Veterinary Medical Database.[b] Only animals that had urinalysis performed and had no urinary tract disease were selected as controls.

The following information was retrieved from urolith submission records of each case animal and medical records of controls when applicable: breed, age, sex, reproductive status (neutered or sexually intact), state of residence, date of urolith submission, anatomic location of the urolith (upper urinary tract [kidneys and ureters], lower urinary tract [bladder, urethra, and voided samples], upper and lower urinary tract, or unknown), and diet (first and second components listed on the record).

Distinction between the Anglo-Nubian and Nubian goat breeds could not be consistently determined from medical records of goats with uroliths submitted to the MUC. Therefore, goats of these 2 breeds were grouped together in the present study. Geographic[j] regions (Southeast, Southwest, Midwest, Northeast, or West) were determined on the basis of state of residence according to classification established by the National Geographic education division[j]. Seasons[i] of sample collection were classified as winter (December, January, February), spring (March, April, May), summer (June, July, August), and fall (September, October, November).

Methods of quantitative urolith analysis – Uroliths for the cases in this study were analyzed by optical crystallography[42-43] and infra-red spectroscopy[79] which were both quantitative urolith analysis methods. In this study only uroliths from cases that were ≥ 70% of the primary mineral were included. Optical crystallography was performed using polarized microscope. In this method trained technicians removed specific crystals from several areas within the sectioned urolith with the aid of a stereomicroscope. The crystals were immersed in oils of known refractive index, and were viewed through a polarizing microscope. Based on the refractive index and crystalline properties, determinations of mineral compositions were made. The polarizing microscopes used in the study included the Olympus BX50 and the Nikon Eclipse 50i polarizing microscopes.

Infrared spectroscopy was used to detect the vibrational characteristics of chemical functional groups. Diffused reflectance infrared Fourier transforms spectroscopy (DRIFTS) and /or Attenuated total reflection (ATR) allowed for determination of composition with mineral sample preparation. The resulting spectrum from either technique was compared with a special library of known compounds to determine the composition of the sample. Nicolet iS10 Infrared Spectroscope from the Thermo-Scientific Corporation was used in this study. There were situations where additional quantative techniques were used when necessary to identify mineral composition of some of the cases. In these scenarios we utilized x-ray crystallography and electron dispersive spectroscopy which was available through collaborating laboratories at the University of Minnesota and elsewhere as was necessary.

Statistical analysis—Standard statistical software [44efgk] was used to determine descriptive statistics (mean, median, and SDs) of breed, age, sex, reproductive status, geographic location of goats, season of urolith submission, and anatomic location of the uroliths within the urinary tract for case animals. Logistics regression was used and ORs and 95% CIs calculated by use of the Woolf method [45] to assess whether breed, age, sex, reproductive status, season, and geographic location were associated with the presence of struvite uroliths. If any expected cell frequency in a contingency table was < 5, the Fisher exact test was used.[46] Breeds were further categorized as African (Pygmy, Nigerian Dwarf, and Boer) or non-African (all others) in origin.

For analysis of age effects, goats were divided into 6 age groups: < 1 year, 1 to < 2 years, 2 to < 4 years, 4 to < 7 years, 7 to < 10 years, and ≥ 10 years. Data for goats < 2 years of age were also compared with those for goats' ≥ 2 years old. Referents for the variables (determined on the basis of breaks in the data and mean age) for determination of ORs were as follows: breed, mixed; breed origin, non-African; age, < 1 year; sex, female; reproductive status, sexually intact; season, spring; and geographic region, Southwest.

Prevalence of struvite uroliths was expressed as the relative frequency of all goat uroliths submitted for analysis to the MUC. Relative frequencies also were used to describe the breed, age, sex, reproductive status, and geographic location of goats, as well as season of collection and anatomic location of uroliths. Logistic regression was computed to determine whether the yearly percentage of goats with struvite uroliths of Africa and no-African origins increased or decreased over the study period. The 28-year study was grouped into 2 intervals (1984 through 1998 and 1999 through 2012) to determine whether risk or protective factors changed over time. The Breslow-Day statistic was computed to determine whether ORs were homogenous over the 2 time intervals.[47] The Mantel-Haenszel summary of ORs was computed when the results of the Breslow-Day test were not significant. Values of $P < 0.05$ were considered significant. Because of the absence of continuous variables, crude ORs were computed by means of hierarchic well-formulated modeling to find the best risk model for the categorical variables of breed, age, sex, reproductive status, geographic location, season, and anatomic location. After adjusting for age, sex, and reproductive status as confounding factors and interactions, risk factors associated with urolith formation were determined from the best model.

Odds ratio estimates were considered significantly different from 1 if the 95% CI did not encompass 1.0.[48] On the basis of recommendations by Lilienfeld and Stolley, [49] we classified associations with significant ORs between 1.1 and 1.9 or between 0.5 and 0.9 as weak. Significant ORs > 2 (indicating a risk factor) and those < 0.5 (suggesting a protective effect) were interpreted as clinically (biologically) important. All analyses were performed with standard software.[44, c, d] Results were considered as significant at values of $P < 0.05$.

RESULTS

Because of the few number of struvite uroliths retrieved from goats that was involved in this study, results of the logistic regression was inconclusive and therefore was not reported here. As a result of this observation only descriptive statistics was reported here. Uroliths from 832 goats were submitted to the Minnesota Urolith Center for analysis during the study period. Of these, uroliths containing ≥ 70 % struvite were only 3%

(n=29). Struvite content of the 29 case goats was as follows: 100%, 15 (52%) samples; 90% to 99%, 5 (17%) samples; and ≥ 70% to 89%, 9 (31%); samples). Struvite uroliths retrieved from goats were typically irregular in shape. They were brownish and whitish in color and range from < 1 mm to > 1 cm in diameter (**Figure 1**).

Breed—Case goats included 7 breeds and control goats included 11 breeds. Goval breed (n=1 case goat) was excluded from breed-related analysis because of lack of control animal. Breed data for 9 of 29 (31%) case and 124 of 16,366 (0.8%) control animals were not provided at the time of urolith submission. Of the 20 struvite submissions where breed was recorded, 14 (70%) and 6 (30%) were breeds of African and non-African origins respectively. Goats of African origin (Pygmy, Boer and Nigerian Dwarf) comprised 70% (n=14) of breeds with struvite uroliths (**Tables 1, 2, & 3**). From 1984 through 1998, case goats of African descent constituted 5 of 7 (71%) of goats with struvite uroliths. Goats of non-African descent constituted 2 of 7 (29%) of goats with struvite uroliths. During the same period, 1,579 of 11,205 (14%) control goats were of African descent, and the remaining 9,626 (86%) were of breeds with other origins. From 1999 through 2012, 9 (69%) and 4 (31%) of 13 case goats were of African and Non-African descent respectively. Among 5,037 control goats seen during this period, breeds of 1,816 (36%), and 3,221 (64%) were of African and non-African origins respectively. Breed origin data were unavailable for 9 case and 124 control goats seen between 1999 and 2012 (**Tables 1 2& 3)**.

Age—The mean age of 23 case goats for which data were available was 1.8 years (range, 3 months to 9.8 years). Seventeen (74%) of the goats were < 2 years and 6 (26%) were ≥ 2 years of age. Goats < 1-year-old constituted 13 (57%); 1 to < 2 years comprised 4

(17%); 2 to < 4 years comprised 3 (13%), and 7 to < 10 years comprised 3 (13%) of the case goats respectively. None of the case goats was up to 10 years of age (**Table 2**).

Sex and reproductive status—Of the 29 case goats, sex was recorded for 28 (97%). Of the 28, all were males, and none was female. Reproductive status for 28 of 29 (97%) case goats was recorded; and of these, 12 (43%) were neutered and 16 (57%) were sexually intact. A higher proportion of sexually intact male goats (16 of 28[57%]) had struvite uroliths, compared to neutered males (12 of 28[43%]; **Table 2**).

Anatomic location—Sites from which the uroliths were retrieved were reported for 28 case goats and unknown for 1. Twenty-seven of 28 (96%) goats had urolith samples obtained from the lower portions of the urinary tract (urinary bladder, urethra, voided uroliths, or some combination of these). Uroliths from 1 (4%) was collected from the upper urinary tract (kidney or ureter). Submitted uroliths were from the bladder only for 9 (32%) goat, and from the urethra only for 10 (36%) goats (**Table 2**). Submission of uroliths from both ureter and kidney at the same time was uncommon.

Seasonal distribution—Collection and submission of uroliths for the 29 case goats took place in fall (n=6[21%]), summer (n=7[24%], winter (n=7[24%]), and spring (n=9[31%]). Struvite uroliths were more likely to be collected and submitted in spring than in other seasons (**Table 2**).

Geographic distribution— Of the 29 case goats uroliths submitted to the MUC, 1 (3%) were from the Northeast, 11(38%) were from Southeast, 2 (7%) from were Southwest, 11 (38%) were from Midwest and West 4 (14%). There are more submissions of struvite uroliths from Southeast and Midwest than from other regions (**Table 2**).

Diet—The primary types of diet were recorded for 28 of 29 (97%) case goats. Eleven of the 28 (39.3%) goats were fed grass hay, alfalfa hay or kept on pasture, 2 (7.1%) were fed Purina diet, 2 (7.1%) were fed sweet feed, 5 (17.9%) were fed grains and pellets, 8 (28.6%) were fed commercial foods (types not specified on the submission farms). In 1 case the type of feed used was not recorded on the submission forms.

DISCUSSION

The result of the present study supported our hypothesis that multiple risk factors such as breed, age, sex, reproductive status, geographic location, and season play a role in the development of struvite uroliths. Our reports were in agreement with reports by other investigators.[3, 37] However; those studies [3, 37] were performed without controls. Instead, generalities they observed were based on uncontrolled empirical observations.

As theorized in urolith formation in other species, it is probable that when several risk factors occur together, their individual effects may be enhanced. Thus, each risk factor or protective factor may play either a limited or a major role in the development or prevention of struvite uroliths. [77] However, case-control studies usually cannot demonstrate that risk factors are the cause of a disease. The case animals in the present study were likely goats, with clinical signs and may not have included asymptomatic animals, such as goats that voided uroliths uneventfully, or those whose uroliths were not collected and submitted to the Minnesota Urolith Center for analysis for various reasons (e.g., goats that died as a result of urinary tract obstruction or goats that were euthanized because of complications). As a result of these limitations, this study may not have fully represented the population of all goats with struvite uroliths.

Goat breeds of African descent (Pygmy, Nigerian Dwarf, and Boer) were overrepresented among those with struvite uroliths (i.e., case animals) in our study. This higher prevalence of struvite uroliths in goat breeds of African descent may be associated with a trend of keeping them as pets. As pets, they may be more likely to receive veterinary care when sick, compared with animals kept for other purposes. The number of goats with struvite uroliths in this study may reflect an increased awareness that uroliths can cause urethral obstruction and increased use of diagnostic methods to detect uroliths such as survey radiography and abdominal ultrasonography in the latter half of the study period. This may also be associated with an increased awareness of the availability of complimentary urolith analysis that influenced submissions made during these 2 periods (1984 through 1998 and 1999 through 2012).[a]

The results of our study indicate that goat breeds of African origin were more frequently with struvite urolithiasis, compared to others breeds (i.e., those of non-African origin). Familial predisposition for urolithiasis has been well documented for some mineral types.[80] For example, genetic mutations causing cystine urolithiasis have been identified in dogs [70] and humans.[71]To the authors' knowledge a genetic predisposition for struvite uroliths in goats has not been explored. In our study, the observation that struvite uroliths more commonly affected goats of African descent, compared with other breeds, may suggest genetic predisposition of these breeds to struvite urolithiasis. Prospective studies evaluating the effects of diet, housing, body condition score, and other potential risk factors in addition to breed are necessary to further investigate a potential predisposition to this type of urolithiasis.

In our study, age was significantly associated with development of struvite uroliths in goats, with goats < 1 year of age having more struvite uroliths, compared to other age group categories. Our results are in agreement with reports by other investigators. There have been no reports of urease-producing microbes that are involved in struvite urolithiasis in goats to the authors' knowledge. So it is most likely that struvite uroliths in goats are not associated with urinary tract infections. Because the population of goats with struvite uroliths involved in this study (n=29) was small, this observation should be verified by evaluating a larger population of clinically affected animals that will fully represent the population of all goats with stuvite uroliths.

Of the 29 uroliths retrieved from goats and classified as struvite in the present study indicated that 15 (52%) were composed of 100% struvite, 5 (17%) were composed of 90% to 99% struvite, and 9 (31%). were composed of ≥ 70% to 89% struvite. The question that remains to be answered is why despite the fact that goat urine is normally alkaline but yet goats do not form a lot of struvite uroliths which normally forms in alkaline urine. This question is answered by investigators who reported that since urinary phosphorus excretion is normally very low in goats because excess phosphorus in goats are absorbed in this species and is then secreted back into the digestive system via saliva and then finally excreted in feces.[115] As a result, phosphorus which is one of the major raw materials needed for struvite uroliths formation (others are magnesium and ammonium) is not made available in sufficient amount to form struvite; and as a result struvite uroliths are not as common in goats as one would have been expected. Further studies are needed to unravel the reason behind these observations.

Our results also indicated that struvite uroliths almost exclusively affected males, (28 of 28 [100%]), compared to females with none recorded. For one goat urolith sample the sex was not recorded in the submission form. Results of our study have depicted that struvite uroliths in goats is an all-male problem because males more frequently show more manifestation of the disease compared to females probably due to their urinary tract anatomical differences. This is likely related to the fact that males' urethras are much longer and narrower than those of females.[82]As urocystoliths pass into the distal end of the male urethra (urethral process); obstructive urolithiasis is a likely consequence. The S-shaped sigmoid flexure located just caudodorsal to the testes is another potential site for uroliths to lodge. It is likely that struvite uroliths are not commonly detected in female goats because the wider and shorter urethra allows uroliths to be passed in the urine stream before they can cause clinical signs.

A higher proportion of case goats (16 of 28 [57%]) were sexually intact compared with neutered goats (12 of 28[43%]). The frequency of finding struvite uroliths were significantly greater for sexually intact than the neutered goats. Given that none of the case animals in this study was female this statement may most appropriately be applied to the males only. This observation is different from what we observed in other uroliths of goats where our observation suggests that neutering may be a risk factor for urolithiasis. This observation may be as a result of the very few struvite uroliths involved in this study (29 cases). One group of investigators generated convincing evidence that early neutering may result in underdevelopment of the urethra and thus predispose adult goats to urethral obstruction.[83]The same group of investigators showed that neutering reduces production of testosterone, which normally aids in the development of urethral lumen. A reduced circulating testosterone concentration is also believed to diminish normal preputial-penile attachments.[83] Because the ages at which case goats were neutered was not provided, we were unable to determine whether there was a significant association between urolithiasis and early neutering. These differences may be due to the few goat cases that were involved in this study. There is need for studies that will involve larger number of case goats to be used to authenticate this observation.

Twenty-seven of 28 (96%) case animals in this study had struvite uroliths retrieved from the lower portions of the urinary tract (**Tables 3**). The predominant location of uroliths in the lower portions of the urinary tract of other domestic animals has been described.[84]In our experience in other species, the development of uroliths in the upper urinary tract is likely underreported, because survey radiography and ultrasonography are often times omitted during initial evaluation of animals with clinical signs of urolithiasis. Clinical signs caused by uroliths in the lower urinary tract (increased voiding frequency, straining to void, and dribbling urine) are more likely to be observed than are clinical signs caused by uroliths located in the upper urinary tract (polyuria and hematuria without obvious signs of pain, which may go unrecognized). In the case animals of the present report, uroliths were more commonly retrieved from the urethra only (10 of 28 [36%]) than from the bladder only (9 of 28 [32%]). These localizations are important when considering dietary modification for dissolution of uroliths. It has been recommended that attempts to dissolve urethral uroliths by dietary modification be avoided.[84] Uroliths lodged in the ureters or urethra cannot be dissolved by medical protocols because urolith dissolution requires sustained contact with urine that has been modified and therefore is undersaturated with lithogenic mineral.[84]

In our study, struvite uroliths were detected in goats residing in the Midwest, Southeast, Northeast and west than did goats in the Southwest. These observations indicate that geographic location may be a risk factor for urolithiasis. Geographic prevalence of urolithiasis has been reported in dogs and humans. [85-86]Our study was not designed to determine the reason for the variations among geographic locations. Further research is needed to assess the factors that contribute to such differences.

Struvite uroliths were more likely to be collected and submitted for case goats during the spring than in fall, summer or winter seasons. The association between season and presence of urolithiasis is in agreement with findings of other investigators, who reported that detection of urolithiasis in cats, sheep, and humans varies with season.[87-89] This may be partly attributable to higher seasonal temperatures observed during the summer and fall (leading to heat-induced water loss) in comparison to winter and spring.[87-89] It is also possible that reduced water intake during the winter season could have led to increased urine concentration. We did not investigate possible associations between struvite urolithiasis and these factors.

Diet has been incriminated as important risks factor for struvite uroliths in goats.[90]In our study; we encountered a lack of detailed information regarding diet composition and variability in the reported diets fed to case goats. Lack of consistency in response to our requests for dietary history prevented evaluation of dietary components as risk factors for this disease. To our knowledge, controlled, blinded clinical studies have not been performed to evaluate associations between most dietary risk factors and urolith formation in goats. Our study was also not designed to explore this factor, and we were unable to test for such associations, although it is likely that diet has a role to play in the pathogenesis of struvite urolithiasis. Most case goats for which diet was reported primarily received alfalfa hay, grass hay or pasture (11 of 28 [38%]). Alfalfa and grass hay have a high calcium-to-magnesium concentration ratio and low phosphorus concentration and are also rich in oxalate. Further prospective studies are needed to define the role played by each of these factors singly and in combination as they relate to struvite urolithiasis.

Healthy goats normally excrete urine that is alkaline (pH range, 7.5 to 8.5). Sheep, horses, rabbits, and guinea pigs also normally excrete alkaline urine. Production of alkaline urine is shared characteristics among these herbivorous species. Most of the plants used to feed goats in this study (e.g., grass, grains, and alfalfa) are associated with alkaline urine production.[91] Although, not investigated in the present study, it is probable that alkaline urine pH is a risk factor for struvite urolithiasis in goats, and this should be investigated in future studies. Although, both struvite and calcium carbonate uroliths are formed in alkaline urine, while goats produce a lot of calcium carbonate uroliths but only produce a very few struvite uroliths. Therefore, further study is needed to substantiate this observation or otherwise.

On the other hand, ferrets produce a lot of struvite uroliths but produce only very little calcium carbonate. The observation of the production of more struvite and less calcium carbonate uroliths in ferrets and production of more calcium carbonate and less struvite uroliths in goats are observations that require further investigations to determine the reasons behind such differences for possible inferences. The primary route of phosphorus excretion in ruminants is via the feces, due to salivary recycling of phosphorus with only a small amount excreted via the renal route compared to monogastric animals such as dogs and cats. This may be the reason why goats and sheep produce less struvite uroliths compared to dogs and cats.

Although information derived from the extensive urolith database compiled by the MUC[a] together with data from the Veterinary Medical Database[b] provides a unique resource for retrospective evaluation of the epidemiological features of this disorder, it is important to note that case-control studies and retrospective case series do not prove cause-and-effect relationships. Additional prospective or interventional studies are needed to prove hypotheses derived from such studies.

Knowledge of the predominant mineral types of uroliths in specific species, 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 goats. Modification of risk factors, where possible, may help to minimize urolith formation, dissolve existing uroliths, and minimize recurrences.

Biases and limitations. Since our data was obtained from only goat's urolith submissions to the MUC, it was not a complete representative of all urolith submissions from all breed, age, sex, season and geographic location of all goat populations. Urethras of male goats are substantially longer and narrower than in females. Also the males have urethral process and sigmoid flexure which are absent in females. All these features may have contributed to more clinical manifestations of urethral obstruction in males compared to females and may have therefore influenced their submissions and selections as cases.

Since the MUC analyses are performed at no cost to veterinarians this may have influenced the rate of urolith submissions to the center. Also because the sample size of cases in this study was not very large we were unable to apply the conclusions obtained from the findings of our study to the whole goat populations.

Uroliths obtained from male goats in this study were retrieved mainly from the lower portion of the urinary tract. Clinical signs are more likely to be observed when uroliths are located in the lower urinary tract than from the upper urinary tract. This may have constituted a form of bias in this study leading to higher number of lower urinary tract uroliths being observed in this study compared with the upper urinary tract location.

Furthermore, this study may have included more clinical cases, and may not have included subclinical cases or cases that may have been euthanized due to urinary tract obstructions; as a result, this may have limited the use of results from our study to fully represent the population of all goats with struvite uroliths and therefore may have constituted a form of bias.

Also higher prevalence of struvite uroliths that were observed in goat breeds of African descent might have been due to the increase in the trend of keeping them as pets. As pets, they may have received more veterinary care when sick compared to goats kept for other purposes. This trend may have influenced the submission of uroliths from this group of goats compared to other breeds of goat that are used for other purposes.

Although information derived from extensive urolith data compiled by the Minnesota Urolith Center provides a unique resource for retrospective study, but because we have limited access to goats with uroliths, we have directed most of our efforts toward the evaluation of the epidemiological features of this disorder. Knowledge of the epidemiology of urolithiasis is essential for constructing effective experimental designs and choosing appropriate cases and controls for conducting clinical trials with meaningful results that may facilitate development of surveillance strategies that result in earlier detection of uroliths in goats.

Table 1—Breed distribution for struvite uroliths retrieved from goats and submitted to the MUC between 1984 and 2012 from USA only.

Breeds	**Number of case goats/%**	**No. of control goats/%**
Pygmy	6 (30)	2,715 (16.7)
Boer	7 (35)	794 (4.9)
Alpine	1 (5)	1,415 (8.7)
Saanen	1 (5)	997 (6.1)
Angora	0 (0)	440 (2.7)
Goval	1 (5)	0 (0)
La Mancha	0 (0)	655 (4)
Mixed	0 (0)	4,214 (25.9)
Nigerian Dwarf	1 (5)	22 (< 1)
Anglo-Nubian or Nubian	3 (15)	3,900 (24)
Oberhasli	0 (0)	120 (< 1)
Toggenburg	0 (0)	970 (5.9)
Unknown	9 (31)	124 (0.8)
Total	**29**	**16,366**
Goats of non-African origin	6 (30)	12,803 (78)
Goats of African origin	14 (70)	3,439 (21)
Unknown	9 (31)	124 (0.8)
Total	**29**	**16366**

Table 2—Summary data for 29 struvite uroliths retrieved from goats and submitted to the MUC between 1984 and 2012 indicating, age, sex, reproductive status, season and geographic location and 16,366 control goats obtained from the VMDB about the same period.

Variables	Description	No. of Case goats/%	NO. of Control goats/%
Age	< 1 year	13 (57)	6,290 (42.2)
	1 to < 2 years	4 (17)	2,289 (15.4)
	2 to < 4 years	3 (13)	3,319 (22.3)
	4 to < 7 years	0 (0)	2,044 (13.7)
	7 to < 10 years	3 (10.3)	631 (4.2)
	≥ 10 years	0 (0)	317 (2.1)
	Unknown	6 (21)	1,476 (9)
	Total	**29**	**16,366**
	< 2years	17 (74)	8,579 (58)
	> 2years	6 (26)	6,311 (42)
	Unknown	6 (21)	1,476 (9)
	Total	**29**	**16,366**
Sex	Female	0 (0)	10,248 (63.4)
	Male	28 (100)	5,921 (36.6)
	Unknown	1 (3)	197 (1.2)
	Total	**29**	**16,366**
Reproductive status	Sexually intact	16 (57)	14,160 (87.6)
	Neutered	12 (43)	2,009 (12.4)
	Unknown	1 (3)	197 (1.2)
	Total	**29**	**16,366**
Season	Spring	9 (31)	5,255 (32)
	Summer	7 (24)	4,297 (26)
	Fall	6 (21)	3,425 (21)
	Winter	7 (24)	3,389 (21)
	Total	**29**	**16,366**
Geographic location	South west	2 (7)	1,768 (11)
	Midwest	11 (38)	9,956 (61)
	West	4 (14)	1,479 (9)
	South east	11 (38)	2,773 (17)
	North east	1 (3)	390 (2)
	Total	**29**	**16,366**

Table 3—Summary data for 29 struvite uroliths retrieved from goats evaluated at the MUC between 1984 and 2012 and comparison with control goats.

Variables	Description	No. of Case goats/%	No. of Control goats/%
Anatomic location	Upper urinary tract	1 (4)	
	Lower urinary tract	27 (96)	
	Unknown	1 (3)	
	Total	**29**	
	Bladder only	9 (32)	
	Urethra only	10 (36)	
	Others	9(32)	
	Unknown	1(3)	
	Total	**29**	
Breed origin	Non-African breeds	6 (30)	12,803 (78)
	African breed.	14(70)	3,439 (21)
	Unknown	9 (31)	124 (0.8)
	Total	**29**	**16,366**
Changes over time			
	1984-1998	10 (34)	11,266 (68.8)
	1999-2012	19 (66)	5,100 (31.2)
	Total	**29**	**16,366**
Breed origin and study period			
1984-1998	1984-1998		
Non-African breeds		2 (29)	9,626 (85.9)
African breeds		5 (71)	1,579 (14.1)
1999-2012	1999-2012		
Non-African breeds		4 (31)	3,221 (63.9)
African breeds		9 (69)	1,816 (36.1)
	Unknown	9 (31)	124 (0.8)
	Total	29	16,366

Figure 1—Photograph of struvite uroliths from 2-year-old male goat. Measurements are shown in centimeters.

CHAPTER 7

Evaluation of the trends in global urolith submissions in goats:

832 cases (1984-2012).

SUMMARY

This cross-sectional retrospective case study evaluated the global shifts in urolith submission data of 832 goats with uroliths. Uroliths were submitted to the Minnesota Urolith Center (MUC) between January 1, 1984 and December 31, 2012.Changes over 28- year study period was divided into two: January 1, 1984 to Demember 31, 1998 and January 1, 1999 to December 31, 2012 and compared.

The results showed that the proportion of $CaCO_3$ uroliths rose significantly in North America from 40% to 45%; but not in Europe and Asia between the two intervals. The proportion of struvite decreased from 8% to 3%, magnesium calcium phosphate apatite decreased from 9%-to 4% and silica uroliths decreased significantly from 20% to 11% in North American. Mixed uroliths decreased from 7% to 3% in North America. Seventy-eight and 68 per cent of goats with calcium carbonate and silica uroliths respectively were between 2 and 7 years-old.

This study has identified that epidemiological studies of urolithiasis are essential for constructing effective experimental designs and selecting appropriate cases and controls for effective conduct of clinical trials with reproducible results in goats. Also associations of breed, age, sex, and reproductive status with urolith type were reported to help veterinarians recognize disease prototypes to improve diagnostic efficiency and to assist researchers identify appropriate goat models for future prospective studies.

INTRODUCTION

Epidemiological studies have been known and proven to be very important tools to be used to identify emerging trends in diseases of human and animal interests. Armed with this tool for instance, Minnesota Urolith Center (MUC) has been tracking the occurrence of uroliths from animals over the past 30 years. Since 1981, quantitative analysis of uroliths from over 1.3 million animals has been performed by the center of which approximately more than 1,000 of them were retrieved from goats.[8, 60, 116] In the month of June, 2015, the MUC celebrated the milestone achievement through the analysis of their 1- millionth urinary stone which came from a 4-year-old neutered male Shih Tzu named Snickers, who was treated at Dogwood Animal Hospital in Fayetteville, North Carolina. Thanks to the thoughtfulness and foresight of the founder and founding director of the MUC, Dr. Carl Osborne and, his team. Through their efforts and support from Hill's Pet Nutrition, the MUC has been able to provide urolith analysis services to veterinarians all around the world at no cost. During this period, the MUC has reported changes in the relative frequencies of different mineral type of uroliths, and the breeds and ages of animals forming them.[8, 60, 116] Furthermore, some authors have erroneously reported that Minnesota Urolith Center analyses only represent uroliths from the USA. While it is true that the majority of the submissions to the center represent stones retrieved from animals residing in North America, however, the Minnesota Urolith Center has analyzed uroliths from animals residing virtually on all the world continents except Antarctica. During this period, the MUC has reported changes in the relative frequencies of different mineral types of uroliths, their location in the globe and the breeds and ages of animals forming them.

The aim of the study reported here was to determine the recent changes in the frequencies of goat urolith types worldwide, while taking into account regional differences. Also associations of breed, age, sex, and reproductive status with urolith type were reported to help veterinarians recognize disease prototypes to improve diagnostic efficiency and to assist researchers identify appropriate goat models for future prospective study.

MATERIALS AND METHODS

Identification of cases and medical records review—Medical records of the MUC[a] were reviewed to identify goats for which urolith samples were submitted by veterinarians within the United States and all around the world between January 1, 1984 and December 31, 2012. The mineral composition of the uroliths was determined by optical crystallography [42, 79] and, when necessary, by infrared spectroscopy .[42, 79] Case animals were identified as goats that had uroliths consisting of ≥ 70% of a single mineral which was identified as that mineral. A urolith without a nidus or shell that contains < 70% of any single mineral was referred to as a mixed urolith. On the other hand, compound uroliths were defined as possessing a central core or outer layer containing ≥ 70% of a single mineral of opposing outer layer or central core of different minerals. To evaluate changes over 28 years' study period, data from two periods were compared; submissions from january1, 1984 to December 31, 1998, and the submissions from January 1, 1999 to December 31, 2012. To maximize submissions, urolith analysis remains at no cost to veterinarians worldwide. The information evaluated from each report included quantitative urolith composition, country and continent of uroliths origin; and breed, sex, reproductive status and age of the goats from which uroliths were retrieved. Because data were collected without identifying the goat and owner, the data set may have included recurrent urolith cases from the same goat. To prevent the effects of such situation, data were tabulated from two fairly short periods so as to reduce this possible overestimation. To evaluate global changes in the frequencies of goat uroliths and different breeds that were involved, urolith submissions to the Minnesota Urolith Center between 1984 and 1998 (n = 133) were compared to submissions that were made between 1999 and 2012 (n =699). The shift in global urolith submissions from goats were evaluated to determine changes over the past 28 years, by comparing data obtained from the submissions for the two portions of the study period.

Methods of quantitative urolith analysis— Uroliths from cases goats in this study were analyzed by optical crystallography[42-43] and when necessary by infra-red spectroscopy[79] which were both quantitative urolith analysis methods. In this study only uroliths from cases that were ≥ 70% of the primary mineral were included. Optical crystallography was performed using polarized microscope. In this method trained technicians removed specific crystals from several areas within the sectioned urolith with the aid of a stereomicroscope. The crystals were immersed in oils of known refractive index, and were viewed through a polarizing microscope. Based on the refractive index and crystalline properties, determinations of mineral compositions were made. The polarizing microscopes used in this study included the Olympus BX50 and the Nikon Eclipse 50i polarizing microscopes. Infrared spectroscopy was used to detect the vibrational characteristics of chemical functional groups. Diffused reflectance infrared Fourier transform spectroscopy (DRIFTS) and /or Attenuated total reflection (ATR) allowed for determination of composition with mineral sample preparation. The resulting spectrum from either technique was compared with a special library of known compounds to determine the composition of the sample. Nicolet iS10 Infrared Spectroscope from the Thermo-Scientific Corporation was used in this study. There were situations were additional quantitative techniques were used when necessary to identify mineral composition of some of the cases. In these scenarios we utilized x-ray crystallography and electron dispersive spectroscopy which was available through collaborating laboratories at the University of Minnesota and elsewhere as was necessary.

Diagnostic criterion and classification of uroliths—Because the outer layer (s) of compound uroliths most of the times are formed as a result of changes in metabolism, disease, medication or diet after formation of the central core, compound uroliths were categorized on the basis of composition of their central core. For instance, a compound urolith with a central core of calcium oxalate and an outer layer of struvite was grouped with other calcium oxalate uroliths and not as a compound urolith. This way of subcategorizing compound uroliths was proposed because it is most likely that if the central core had not been present to serve as a framework for epitaxy, the outer layers would not have been formed in the first place.

On the basis of mineral composition, in this study, uroliths were grouped into the following categories: calcium oxalates which consisted of monohydrate or dehydrate salts of calcium oxalate. Calcium carbonate, struvite, silica, calcium phosphate apatite, calcium phosphate carbonate, magnesium calcium phosphate apatite, and magnesium calcium phosphate carbonate were categorized individually. Compounds consisted of a central core or outer layer containing $\geq$ 70 per cent of a single mineral with an opposing outer layer or central core of a different mineral were classified as individual urolith. The category, 'other' consisted of drug metabolites, and other uroliths of rare or unusual composition were classified as a group.

Statistical analysis—Standard statistical software [44efgk] was used to determine descriptive statistics (mean, median, and SDs) of breed, age, sex, reproductive status, and geographic location of the goats from which the uroliths were retrieved. The proportion of urolith types in each study period was calculated in relation to continent of origin, breed, age, sex, and reproductive status of the goats from which the uroliths were retrieved. Statistical differences between proportions were assessed with a comparison of two proportions. Odds ratios and logistic regression were calculated at 95% CIs using the Woolf method. Results were considered as significant at values of $P < 0.05$. Urolith prototypes were constructed to represent the most common breed, and sex, and the mean age of goats affected by a particular mineral type.

RESULTS

Between January 1, 1984 and December 31, 1998, the MUC analyzed uroliths from 133 goats residing in 3 continents of Asia, Europe and North America. No uroliths were received from Africa, Australia-Oceania, and South America. To identify changes if any, these results were compared with 699 goat urolith submissions received 14 years later between January 1, 1999 and December 31, 2012. In 1984-1998 a prototypical goat with urolith worldwide was a male (96%) with a mean age of 2.5± 1.8 years, goat of African origin (Pygmy; 40%), and neutered male (96%). The breed, sex and age were not recorded on the submissions for 14%, 2% and 16% of case goats, respectively. The most common urolith at this period was calcium carbonate ([40%]; **Table 4**). Other mineral types that were identified during the period included silica (20 %), calcium phosphate apatite (10 %), struvite (8 %) and magnesium calcium phosphate apatite ([9 %]; **Table 4**). Fourteen years later, the prototypical goat with uroliths was a male (95%), still goat of African origin ([Pygmy]; 39 %), neutered (74 %); and a mean age of 3± 2.3 years. The breed, sex, and age were not reported in the submission forms for 25%, 3% and 14% of the case goats respectively. On contrasting with the previous study period, the most common urolith during 1999-2012 was still calcium carbonate (45%). Some of the other uroliths retrieved from goats at the same study period were magnesium calcium phosphate carbonate (25 %) and silica ([12 %]; **Table 4**).

Uroliths retrieved from goats and submitted to the MUC rose from 16% (n= 133) in 1984-1998 to 84% (n=699) in 1999-2012 world-wide. During this period also, the proportion of urolith submissions from North America has increased from 16% in 1984-1998 to 83% in 1999-2012, while submissions from Europe increased from zero per cent to 0.9% and in Asia it rose from zero per cent to 0.3 % **(Table 5).** In 1984-1998 world-wide core mineral submissions were calcium carbonate 40%, calcium phosphate apatite 10%, struvite 8%, magnesium calcium phosphate apatite 9%, and silica 20%. The Mixed and compound uroliths as well as other materials comprised of 7%, 3% and 0.8% respectively. In 1999-2012 the primary minerals retrieved from goats were calcium carbonate 45%, compound 6%, magnesium calcium phosphate apatite 3%, magnesium calcium phosphate carbonate 25%, mixed 3%, silica 12%.and matrix 0.6% **(Table 5).**

Calcium carbonate— In 1984-1998, $CaCO_3$ was the most common urolith and continued to lead the way in 1999-2012. The proportion of $CaCO_3$ submissions had increased from 40% in 1984-1998 to 45% in 1999-2012 which was fourteen years later ($P< 0.001$; **Table 5**). No $CaCO_3$ urolith was submitted from Europe in 1984-1998 but 6 $CaCO_3$ uroliths were submitted from Europe in 1999-2012. No calcium carbonate urolith was submitted from Asia during the two study periods. In 1984-1998, the prototypical goat with $CaCO_3$ uroliths has a mean age of 4±2.8 years and fourteen years later in 1999-2012 the mean was 3.9 ± 4 years. Atypical goat with $CaCO_3$ in 1984-1998 was a male (98%) of which (96%) were neutered, goat breeds of African descent (46%; Pygmy breed). In 1999-2012, the prototypical goat with $CaCO_3$ was a male (95%) of which (74%) were neutered), and goats of African descent (47%; Pygmy breed; **Table 5**). In 1999-2012, the most common goat breeds with $CaCO_3$ urolithiasis included Boer (11%), Pygmy (30%), Nigerian Dwarf (5%), Saanen (6%), Nubian (8%) and Alpine (6%). Worldwide, these breeds comprised 66% of all $CaCO_3$ urolith submissions during the study period. Furthermore, 89% of all $CaCO_3$ goats were between the ages of 2 and 10 years. During this period; less than 11% of goats with $CaCO_3$ uroliths were less than 2 years of age.

Silica— In 1984-1998, all goat silica uroliths submitted to the MUC were received from North America. During this period, 20% of all goat urolith submissions were silica. Fourteen years later, the proportion of silica urolith submission had decreased significantly to 12% ($P = < 0.001$; **Table 4 & 5**). Continent by continent, silica was completely absent in Asia and Europe, but a significant decrease was observed in North America. In 1984 -1998, a prototypical goat with silica was male (96 %) of which 94% were neutered, goat of African descent (Pygmy; 39%); with mean age of 2.5± 3.1 years and the age range of the affected goats were from 2 months to 12.3 years (**Table 1 & 2**).

In 1999-2012, the prototypical goat with silica urolith was male (95%) of which (87%) were neutered, goat breeds of African origin (Pygmy; 28%), with mean age of 3.4 ± 2.3 years. and the age range was from 2 months to 10 years (**Table 1 & 2**). The results of our study indicated that silica urolith submission decreased significantly world-world-wide ($P < 0.001$; **Table 5**).

Struvite—In 1984-1998, 8% of goat urolith submissions were struvite. In 1999-2012, the per cent of struvite had decreased to 3 per cent. In 1984-1998, the prototypical goat with struvite was male (96%) of which (96%) were neutered, goat of African descent (Boer breed 50%); with a mean age of 1 year, and age range of 3 months to 2 years. In 1999-2012, the prototypical goat with struvite was male (100%) of which (96%) were neutered, goat of African descent (Boer, 50%), with mean age of 2.5± 1.8 years and ranged from 3 months to 7 years (**Table 1, 2, 4& 5)**.

Magnesium calcium phosphate carbonate—In 1984-1998, 0.2 per cent of goat urolith submissions was magnesium calcium phosphate carbonate. In 1999-2012, the per cent had increased to 25 per cent (**Table 5**). In 1984-1998, the prototypical goat with magnesium Calcium Phosphate Carbonate was a male (100%) of which (100%) were neutered, goat of African descent (pygmy; 50%). The age of the 2 goats with magnesium phosphate carbonate uroliths was not recorded for the 1984-1998 study period. In 1998-2012, the prototypical goat with magnesium calcium phosphate carbonate urolith was male (96%) of which (100%) were neutered, goat of African descent (Pygmy; 39%), with mean age of 1.4± 1.6 years. Only 1 magnesium calcium phosphate carbonate urolith was submitted from Asia, none at all was submitted from Europe (**Tables 2, 4 & 5**).

Magnesium calcium phosphate apatite—In 1984-1998, 9 per cent of goat urolith submissions was magnesium calcium phosphate apatite. In 1999-2012, the per cent had decreased significantly to 4 per cent ($P < 0.001$; **Table 5**). In 1984-1998, a prototypical goat with magnesium calcium phosphate apatite was male (100%) of which (96%) were neutered, goat of African descent (Pygmy; 50%), with mean age of 0.6 months. All the affected case goats were from North American (**Table 5**). The breed for one of the case goats was not recorded. In 1999-2012, a prototypical goat with magnesium calcium phosphate apatite was male (100%) of which (45%) were neutered, goat of African descent (Pygmy, 42%), with mean age of 1.8 ± 2.3 years, and a range of 1 month to 9 years. During this period 98 per cent of the uroliths retrieved from goats and submitted to the MUC were received from North America. On the other hand, urolith submissions retrieved from goats and submitted from Asia and Europe were 0.3% and 0.9% respectively (**Table 5**).

Compound—In 1984-1998, 3 percent of goat urolith submissions was compound. In 1999-2012, the per cent increased to 6 per cent (**Table 5**). In 1984-1998, a prototypical goat with compound uroliths was a male (100%) of which (100%) were neutered, goats of African origin (Pygmy, 40%), with mean age of 1.9 ± 2.3 years. Worldwide, three breeds of goat, (Pygmy, Nigerian Dwarf and Boer) comprised more than 46 per cent of all compound urolith submissions during this period. In 1999-2012 period, a prototypical goat with compound urolith was male (95%) of which (83%) were neutered, goat breeds of African descent (Pygmy; 28%), with mean age of 3.9±2.3 years, and age range of 5 months to 9 years. All the compound uroliths retrieved from goat and submitted to the MUC during this later period were received from North American. Worldwide, three breeds, (Pygmy, Nigerian Dwarf and Boer) comprised more than 47 per cent of all compound urolith submissions retrieved from goat during this period (**Tables 5, 2 & 3**).

Mixed Urolith—In 1984-1998, 7 per cent of urolith submissions retrieved from goat was mixed. In 1999-2012, the per cent decreased to 3 per cent (**Table 5**). In 1984-1998, a prototypical goat with mixed uroliths was male (100%) of which (78%) were neutered, breed of African descent (Pygmy 33%), with mean age of 2 ±3.2 years, and a range of 2 months -3 years (**Table 5).** In 1999-2012, the prototypical goat with mixed uroliths was a male (96%) of which (67%) were neutered, goat of African descent (Pygmy; 53%), with a mean age of 2 ±1.6 years, and a range of 3 months-5.5 years. Also all the case goat uroliths submitted to the MUC was received from North American (**Table 5**).

Calcium oxalate (monohydrate and dehydrate)—Calcium oxalate urolith was not a very common finding in goats in this study. Calcium oxalate urolith was zero in 1984-1998 and was observed to be only 1 per cent in 1999-2012 (**Table 5).** All the calcium carbonate uroliths retrieved from goats and submitted to the MUC were received from North America. The mean age of goats with calcium oxalate monohydrate in 1999-2012 was 6 ± 1.9 years and 4.4 ± 1.3 years for calcium oxalate dehydrate uroliths during the same period. The prototypical goat with calcium oxalate dehydrate was male (100%) of which (100%) were neutered, Pygmy goat (100%**)**, and an age range of 7 months to 8 years. A prototypical goat with calcium oxalate monohydrate uroliths was male (100%) of which (100%) were neutered, with a mean age of 6 ± 1.9 years. All the calcium oxalate uroliths retrieved from goat and submitted to the MUC were received from North American. The ages of case goats with calcium oxalate urolith retrieved from goat in 1984-1998 were not recorded in the submission form (**Tables 5 & 2**).

Calcium phosphate apatite —In 1984-1998, 10 per cent of goat urolith submissions to the MUC world-wide were calcium phosphate apatite and this observation decreased to zero per cent in 1999-2012 period (**Table 5).** The mean age of goats with calcium phosphate apatite during 1984-1998 study periods was 0.8 months (**Table 2**). In 1999-2012, the prototypical goat with calcium phosphate apatite was male (100%) of which (96%) were neutered, Nubian breed (58%), with a mean age of 5 ± 2.3 years. All calcium phosphate apatite uroliths retrieved from goats and submitted to the MUC at this interval were received from North American (**Table 5**).

Calcium phosphate carbonate—In 1984-1998, 0.8 per cent of goat urolith submissions to the MUC were calcium phosphate carbonate and 14 years later the proportion came down to 0.1 per cent (**Table 5**). In 1984-1998, the prototypical goat with calcium phosphate carbonate was male (100%) of which (100%) were sexually intact, goat of African descent (Pygmy; 100%), with mean age of 1 year. In 1999-2012, a typical goat with calcium phosphate carbonate uroliths was male (100%) of which (66%) were neutered, Nubian (50%), and the mean age of goat with calcium phosphate carbonate could not be determined because the ages were not recorded in the submission form (**Table 2**). All goat calcium phosphate carbonate urolith submissions were received from North American (**Table 5**).

Others—In 1984-1998, < 1% of goat urolith submissions were classified as other and this included dried blood. They were detected in males (100%) of which (100%) were intact and age was not recorded in the submission form, so we could not determine the mean age of the affected case goats (**Tables 5 & 2**). The breed mostly at risk was Alpine. In 1999-2012, < 1% of urolith submissions from goats were classified as others (**Table 5**). They were observed in Boer breed (100%) of which 100% was neutered. The mean age was 5 ± 4.1 years, and age range of 11 months to 12 years (**Table 2**).

Matrix—In 1984-1998, matrix submissions from goat was zero per cent, but was observed to be < 1 per cent in 1999-2012 (**Table 5**). The mean age of goats with matrix could not be determined because the ages were not recorded in the submission form. (**Table 2**). In 1999-2012, the prototypical goat with matrix was male (100%) of which (100%) were neutered, Nubian breed (100%), with a mean age of 6 ± 2.4, and age range of 4 years to 8 years. All the matrix submissions retrieved from goats and submitted to the MUC were received from North America (**Table 2 & 5**).

Xanthine—Uroliths minerals containing xanthine was not a very common feature seen in goat throughout the study. In 1984-1998, < 1 % of uroliths retrieved from goats and submitted to the MUC was classified as xanthine. No urolith retrieved from goats was received during the period of 1999-2012 (**Table 5**). In 1984-1998, the prototypical goats with xanthine uroliths was male (100%) of which (100%) were intact, Nubian breed (100%), with a mean age of 6 ± 2.4 months, and ranged from 4 to 8 years. The only xanthine urolith submissions retrieved from goat during the whole study period (n=1) was received from North America (**Table 5 & 2**).

Discussion

The results of our study are in agreement with observations made by other investigators who observed that $CaCO_3$ uroliths continue to gain prominence as an emerging problem in goats' population worldwide. [8] In 1984-1998, $CaCO_3$ uroliths comprised 40% of all goat uroliths submitted to the MUC world-wide. In 1999-2012 goat urolith submissions classified as $CaCO_3$ surpassed that of 1984-1998 to 45% world-wide **(Table 5).** Similarly increases in the prevalence of $CaCO_3$ uroliths have also been observed in moose, wildebeests, cows, horses and sheep. [8]

Studies designed to tackle these myriads of etiological risk factors responsible for the increase in $CaCO_3$ urolith formation is essential to reverse this growing trend. Our study identified some key factors likely to improve study design. Calcium carbonate uroliths were diagnosed primarily in goats between the ages of 2 and 7 years. Although $CaCO_3$ uroliths were identified in 17 of more than 600 known breeds of goats, our study is in agreement with other investigators who have observed that only a few breeds, particularly breeds of African origin, were at increased risk for $CaCO_3$ urolithiasis.[37, 117] As a result studying young goats or goat breeds unlikely to form $CaCO_3$ uroliths may provide at a least misleading result contrary to studies evaluating goats mainly at high risk for $CaCO_3$ uroliths development.[37, 117] Evidence that proved this hypothesis was a study evaluating thiazide diuretics to minimize urine calcium excretion. Thiazide was found to be ineffective in healthy beagles, but yet thiazide administration resulted in significant reduction in urine calcium excretion in breeds with spontaneous calcium oxalate uroliths formation. [118]

Other investigators have shown that individual dog breeds provide ideal opportunities for studying genetic determination of disease because compared with human, dogs within specific breed have relatively little genetic diversity, and disease traits are often times controlled by a small number of loci with a strong effect. [119]Based on this premise, goats of African descent may possibly provide an ideal model for exploring the genetic determinants and gene-environment interactions that is responsible for $CaCO_3$ urolithiasis.

Furthermore, for these and other comparison studies, controls have often been non-stone formers of the same breed, age, and sex so as to minimize variability between groups. [120-121]This model is appropriate because these controls represent the population at risk. However, the trade-off is that controls, originally presumed to be stone-free, may form $CaCO_3$ uroliths later in life.

The result of our study with $CaCO_3$ uroliths indicated that urolith formation wanes with age. Based on this premise, goats of African origin may provide an ideal model for studying the genetic determinants and gene environment interactions that influence $CaCO_3$ urolithiasis.

Results of our study were in agreement with other investigators who have reported that the relative per cent of struvite urolith is decreasing globally.[122-125] A variety of factors may have been responsible for this trend. Top on the list is the use of a low levels of ammonium chloride that are routinely included in commercially available goat feed at low levels as a prevention against struvite urolithiasis. This prevention method is expected to create an acidic environment of < pH 6.5 in which struvite uroliths cannot form. This is because increased dissolution rate of struvite uroliths have been observed at a lower urine pH. The production of acidic urine pH in continuous low level ammonium chloride regimen (long term) is short lived due to re-alkalization that is thought to be a physiological response to chronic acidification. [3, 126]

Re-alkalization has also been observed in beef cattle, sheep, cat and rats as has been observed in goats. What this may mean to the clinicians is that the long term continuous ammonium chloride treatment in goats is not effective because it leads to physiological response which will return the urine pH towards alkaline. In addition, long term administration of ammonium chloride regimen has been associated with bone reabsorption and metabolism leading to decreased bone density, and associated fractures, osteopenia and osteoporosis.[3, 126]

The Minnesota Urolith Center advocates for medical dissolution of struvite uroliths save those causing urinary tract obstruction in dogs and cats. Struvite uroliths have been shown to dissolve in acidic urine, both in vitro and in vivo in dogs, cats and rats. The consequence of successful dissolution of struvite is that these uroliths would not be available for analysis; therefore, they would not be included in the numerical count of uroliths analysis report. Presently, there is no consensus on the length of time necessary to dissolve struvite uroliths in goats using acidified urine on naturally obtained uroliths. In addition, long term use of ammonium chloride may also lead to increased renal excretion of calcium which may lead to increased risk for calcium containing uroliths such as calcium oxalate, calcium carbonate, calcium phosphate apatite, calcium phosphate carbonate, magnesium calcium phosphate apatite, and magnesium calcium phosphate carbonate uroliths.[3, 126, 127]

The results of our study is in agreement with this observation because as struvite uroliths decreased we equally observed increases in some calcium containing uroliths in our study from 1984-19987 and 1999-2014 (**Table 5**). Some group of investigators have shown how to prevent this undesirable effect (re-alkalization) associated with long term struvite urolith prevention in goats using low dose ammonium chloride inclusion in feeds that results only in short term urinary acidification which have been clinically proven to be effective for long term use in goats. Because re-alkalization is common in urine of goats receiving continuous ammonium chloride treatment, this method is not effective when used for a long term prevention of struvite urolithiasis in goats.

On the other hand, pulse-dose regimen treatment method has been proven to be very effective in producing a urine pH level below the target range for struvite dissolution (< pH6.5) for long period of time.[3, 126] As a result, pulse- dose regimen of ammonium chloride treatment has been observed to maintain effectiveness at production of acidic urine pH < 6.5 for long term prevention of urolithiasis in goats. In goats with struvite uroliths, the use of diets with reduced magnesium and phosphorus which promotes acidification of urine is essential for prevention of urolith recurrences in this species. However, urine acidification is a risk factor for calcium containing uroliths such as calcium oxalates. Therefore, caution is the key when advocating the use of this method for control of struvite urolithiasis in goats.

Therefore, we hypothesize that some dietary strategies designed to prevent struvite uroliths, may indirectly decrease the occurrence of struvite urolithiasis while increasing the occurrence of calcium containing uroliths. It is interesting to note that the most common breeds that form struvite uroliths were the same breeds that form calcium containing uroliths (i.e., calcium carbonate, calcium phosphate carbonate, calcium phosphate and calcium oxalate).[122] In this study, 8% of urolith submissions in 1984-1998 were struvite but this observation had decreased to 3% in 1999-2012. In 1984-1998, a prototypical goat with struvite urolith was male (100%) of which (37%) were neutered, goat of African descent (pygmy breed, 50%), with mean age of 1month, and range of 3 months to 2.2 years **(Table 2 & 5**).

In 1998-2012, the prototypical goat with struvite urolith was a male (100%) of which (62%) were neutered, also goat of African descent (Boer, 50%), with mean age of

2.5 years and a range of 3 months to 7 years (**Tables 2 & 5).** We also observed that compared with goats with struvite uroliths, the mean age of goats with calcium containing uroliths were older and the proportion of the males were higher. As a result of these observations, we hypothesize that several interacting mechanisms account for the change in trends in urolith types in breeds at risk for struvite and calcium containing uroliths. Firstly, these breeds are more likely to possess common structural as well as functional risk factors predisposing them to several types of uroliths. Secondly, while in dogs and humans struvite urolithiasis is associated with a urinary tract infection, however, to our knowledge there are no reports of infection-induced struvite urolithiasis in goats. As a result, high urinary solutes and alkaline urine pH resulting from dietary mineral imbalances as well as high mineral and carbohydrate contents are likely responsible for struvite urolith development in goats. Because calcium containing uroliths occur in older goats, the clinical implication for this is that the practice of acidifying urine to prevent struvite uroliths would be contra-indicated in these breeds as they get older especially in males. Also acidification of urine promotes urine calcium excretion by increasing mobilization of calcium from bones, increasing filtration of ionized calcium from ultrafiltration which is associated with bone reabsorption and metabolism leading to decreased bone density, and associated fractures, osteopenia and osteoporosis. [128] Increased urine calcium with aging has also been observed in humans. [129]

The results of our study indicate that silica urolith submissions to the MUC world-wide decreased from 20% between 1984 and 1998 to 12% between 1999 and 2012. This decrease specifically referred to North America because all the silica uroliths received during the two periods came from that continent.   Current epidemiological reports indicate that silica uroliths remain uncommon.[124, 130] One exception to this generality was a report on dogs in Mexico City in which the reported proportion of silica uroliths was 14%. [113] According to the authors, the high concentration of silica in drinking water in Mexico were likely responsible for this observation. It is a common knowledge that dietary factors are important risk factors for silica uroliths formation. However, our study indicated that silica uroliths were uncommon globally because all the case goats in this study originated from North America. Since the Minnesota Urolith Center comparatively received very few to almost zero uroliths for analysis from South American, our data may not have provided a suitable comparison for the distribution of silica uroliths affecting goats in that geographic location. So we take this information as stated without having to compare this observation with our results because of the above stated reasons.

The proportions of calcium phosphate apatite, calcium phosphate carbonate, and magnesium calcium phosphate apatite had declined from 9.8%, 0.8%, and 9% between 1984 and 998 to zero per cent, 0.1% and 4% between 1999 and 2012 respectively in North America. A slight increase was observed in magnesium calcium phosphate apatite in Asian between 1999 and 2012 (**Table 5**). None of the above mentioned minerals were submitted from Europe during the stated time period.

Calcium oxalate uroliths in this study was only received from North America throughout the 2 study periods. Calcium oxalate urolilths increased from zero per cent between 1981 and 1998 to 1%, between 1999 and 2012. Struvite decreased significantly (< 0.001) from 7.5% between 1984 and 1998 to 2.7% between 1999 and 2012 in North America. Calcium oxalate has been equally increasing worldwide while struvite is decreasing world-wide in dogs and cats.[123] Struvite urolithiasis in animals has been routinely treated with reduced magnesium and phosphorus that promotes acidification of urine which is essential in preventing urolith recurrence .[131] However, urine acidification is a risk factor for calcium oxalate uroliths formation .[131] Based on this premise, we therefore hypothesize that some dietary strategies to prevent struvite urolithiasis may indirectly decrease the occurrence of struvite while increasing the occurrence of calcium oxalate. Because the amount of struvite and calcium oxalate uroliths involved in this study were very few we were unable to determine whether there was a reciprocal relationship between these two minerals as observed in dogs and cats.

In this study the same goats breeds (breeds of African origin) which most commonly form struvite uroliths were the same breeds that form calcium containing uroliths (i.e. calcium carbonate, calcium oxalate, calcium phosphate apatite, calcium phosphate carbonate, magnesium calcium phosphate apatite and magnesium calcium phosphate carbonate) which is in agreement with other investigators .[122] In this study, pygmy breed accounted for 40% of calcium carbonate, 10% of calcium phosphate apatite, 8% struvite, 9% magnesium calcium phosphate apatite and 2% magnesium calcium phosphate carbonate of all urolith submissions between 1984 and 1998 (**Table 5**).

Compared with goats with struvite uroliths, the mean age of goats with calcium – containing uroliths was older and the proportion of males was higher (**Table 2**). We hypothesize that several interacting factors account for the shifts in the urolith types in breeds at risk for struvite and calcium containing uroliths. Firstly, these breeds most likely possess common denominator and/ or functional risk factors predisposing them to several types of urolith. Secondly, young adults are at increased risk for developing urinary tract infections with urease producing microbes (staphylococcus). In a report from one study evaluating the epidemiology of urinary tract infection in dogs with urolithiasis, the mean age of females (6.24 years, n=235) and males (9.08 years, n=794) with E coli positive cultures.[132]

The higher proportion of bacterial urinary tract infections in female dogs was a result of the higher proportion of female dogs with infection-induced uroliths (e.g. struvite, and calcium phosphate carbonate). As a result of this we hypothesize that urine calcium in adult goats' increases with age. This report is different from our study results in which goats with calcium phosphate carbonate uroliths were younger while in the former they were much older (**Table 2**). Calcium phosphate carbonate is an infection-induced uroliths caused by the same urease-producing bacteria as in struvite urolithiasis. Under a similar situation, increased urine calcium appears to initiate the formation of calcium phosphate carbonate. Increased urine calcium with aging has also been reported in human.[21]Similarly, association between ageing with fewer urease-producing urinary tract infections (reported in dogs) and more urine calcium excretion, supports the sex shift to males that is recognized in calcium carbonate urolith-formers in our study.

If this hypothesis is true as stated, the clinical drawbacks of these shifts is that the practice of acidifying urine to prevent struvite uroliths formation will be contraindicated in these breeds as they grow older. This recommendation was chosen because hypercalcemia is a regular abnormality in calcium oxalate -forming dogs [121], and because acidification of urine promotes urine calcium excretion by increasing mobilization of calcium from bone, thereby increasing filtration of ionized calcium from blood and decreasing renal tubular reabsorption of calcium from utrafiltrate.[121]

Mixed uroliths were submitted from goats residing only in North America and none from Asia and Europe during the two study periods. Therefore, mixed urolith detection is not a world-wide problem in goats as per the results of our study. Even in North America where it was recorded, the submission of mixed uroliths decreased from 7% in 1984-1998 to 3% in 1999-2012 (**Table 5**). We were unable to determine the reason for the decline in mixed urolith submissions from North America or the reason for the complete absence of mixed uroliths from goats residing in other continents.

Compound uroliths were also only submitted from goats residing in North America during the two periods. In North America the submission of compound uroliths increased from 3% between 1984 and 1998 to 6% between 1999 and 2012. We were unable to determine the reason for this increase in this mineral type (**Table 5**).

The group of uroliths classified as other in this study (which included dried blood, drug metabolites, and uroliths of rare or unusual composition) were not commonly seen in goat uroliths. This group of uroliths were only retrieved from goats residing in North America. The only increase observed in this mineral group was very minimal and was from 0.8% between 1984 and 1998 to 0.9% between 1999 and 2012 in North America **(Table 5)**. We were unable to determine the reason for the rarity of this type of mineral worldwide.

Xanthine urolith is a rare mineral seen in goat uroliths worldwide in this study. The only xanthine urolith (n=1) from goat submitted to the MUC during the study period was received from North America between 1984 and 1998. This xanthine urolith submission received from North America comprised only 0.8% per cent of all the uroliths submitted during the said interval. No xanthine uroliths from goats residing either in Asia or Europe were received during the same study period. We were unable to determine the reason for the rarity of xanthine uroliths retrieved from goats world-wide.

Inclinations favoring ownership of goat breeds highly at risk for urolithiasis may have contributed to the worldwide shifts in different urolith types. Such inclinations included but not limited to the increased use of goats of African descent as household pets. However, subsequent to the short time-span in which sweeping changes in the proportion of some types of uroliths occurred, other risk factors may also have been involved. It is the authors' opinion that changes in husbandry, pet uses, and nutrition represent significant contributing risk factors responsible for these epidemiological shifts. Subsequently, appropriate changes in husbandry and nutrition as well as the choice of a particular breed of goats as pets may potentially provide the needed solutions for minimizing the disease stress condition.

Biases and limitations. Since our data was obtained from the same pool of uroliths submitted to the MUC, it may not have been a complete representative of all urolith submitted from every goat everywhere.

Urethras of male goats are substantially longer and narrower than females. Also the males have urethral process and sigmoid flexure which are absent in females. All these features may have contributed to more clinical manifestations of urethral obstruction in males compared to females and may have therefore influenced their selection as cases.

Also since the Minnesota Urolith Center uroliths analyses are performed at no cost to veterinarians world-wide this may have influenced the rate of urolith submissions to the center. Because the sample size of uroliths in this study was not very large we were unable to apply the conclusions drawn from our findings to the whole goat population. Uroliths obtained from male goats in this study were retrieved from the lower portion of the urinary tract. Clinical signs are more likely to be observed when uroliths are located in the lower portions of the urinary tract than from the upper portions. This may have constituted a form of bias in this study leading to higher number of lower urinary tract uroliths being observed in this study compared with upper urinary tract.

This study may have included more clinical cases, and may not have included subclinical cases or cases that may have been euthanized due to urinary tract obstructions; as a result, this may have limited the use of results from our study to fully represent the population of all goats with uroliths world-wide. As a result, this may have constituted a form of bias in our study.

Higher prevalence of uroliths observed in goat breeds of African descent may have been due to an increase in the trend of keeping them as pets. As pets, they may have received more veterinary care when sick compared to goats kept for other purposes. This trend may have influenced submission of uroliths from this group of goats compared to other breeds of goat that are used for other purposes.

Although information derived from extensive urolith data base compiled by the Minnesota Urolith Center provides a unique resource for retrospective study, but because we have limited access to goats with uroliths, we have directed our efforts toward the evaluation of the epidemiological features of this disorder. Knowledge of the epidemiology of urolithiasis in goats is essential for constructing effective experimental designs and choosing appropriate cases and controls for conducting clinical trials with meaningful results that may facilitate development of surveillance strategies that result in earlier detection of uroliths in goats.

Table 1—Global shifts in age (mean & range), sex, reproductive status and geographic location of goats with uroliths submitted to the MUC (1984-1998) VS (1999-2012).

Variables	**1984-1998**	**1999-2012**	**Total Number/%**
Sex			
Male	128 (96.2)	665 (95.1)	793 (95.3)
Female	2 (1.5)	13 (1.9)	15 (1.8)
Unknown	3 (2.3)	21 (3)	24 (2.9)
Total	**133 (100)**	**699 (100)**	**832 (100)**
Reproductive status			
Sexually intact	2 (1.5)	162 (23.2)	164 (19.7)
Neutered	128 (96.2)	516 (73.8)	644 (77.4)
Unknown	3 (2.3)	21 (3)	24 (2.9)
Total	**133 (100)**	**699 (100)**	**832 (100)**
Geographic distribution			
Asia	0 (0)	2 (0.3)	2 (0.2)
Europe	0 (0)	6 (0.8)	6 (0.7)
North America	133 (100)	691 (98.9)	824 (99.1)
Total	**133(100)**	**699(100)**	**832 (100)**
Age			
Minimum age	2 months	2 months	
Maximum age	12.3 years	14.7 years	
Age range	2 months-12.3 years	2 months -14.7 years	
Mean age	2.5 years	3 years	
Unknown	21 (15.8%)	98 (14%)	

Table 2— Changes in the global mean age of goats with different urolith minerals: (1984-1998) VS (1999-2012)

	1984-1998	1999-2014
Mineral	**Mean age / yr.**	**Mean age/yr.**
Calcium carbonate	4	3.9
Calcium phosphate apatite	0.8	4.6
Calcium phosphate carbonate	1	NA
Compound	1.9	3.9
Magnesium ammonium phosphate 6 hexahydrate	1	2.5
Magnesium calcium phosphate apatite	0.6	1.8
Magnesium calcium phosphate carbonate	NA	1.4
Mixed	1.7	2
Silica	2.5	3.4
Calcium oxalate monohydrate	NA	6
Calcium oxalate dehydrate	NA	4.4
Matrix	NA	6
Other	NA	5.3

Table 3—Global urolith submission shifts in from different breeds :(1984-1998) compared to (1999-2012)

	1984-1998	1999-2012	
Breed	**Number/%**	**Number/%**	**Total Number/%**
Fainting	2 (1.5)	5 (0.7)	7 (0.8)
Alpine	8 (6)	38 (5.4)	46 (5.5)
Nubian	20 (15)	43 (6.1)	63 (7.6)
Angora	5 (3.8)	15 (2.1)	20 (2.4)
Arcot	1 (0.8)	0 (0)	1 (0.1)
Boer	6 (4.5)	91 (12.8)	97 (11.7)
Oberhasli	0 (0)	9 (1.3)	9 (1.1)
Mixed	6 (4.5)	17 (2.4)	23 (2.8)
Pygora	0 (0)	3 (0.4)	3 (0.4)
Cashmere	0 (0)	2 (0.3)	2 (0.2)
Nigerian Dwarf	2 (1.5)	40 (5.7)	42 (5)
LA Mancha	1 (0.8)	12 (1.7)	13 (1.6)
New Zealand kiko	1 (0.8)	0 (0)	1 (0.1)
Pygmy	53 (39.8)	198 (28.3)	251 (30.2)
Saanen	6 (4.5)	43 (6.2)	49 (5.9)
Toggenberg	2 (1.2)	12 (1.7)	14 (1.7)
Unknown	20 (14)	171 (24.5)	191 (22.9)
Total	**133 (100)**	**699 (100)**	**832 (100)**
Breed category			
African breeds	61 (45.9)	329 (47.1)	390 (46.8)
Non-African breed	52 (39.1)	199 (28.5)	251 (30.2)
Unknown	20 (15)	171 (24.4)	191 (23)
Total	**133 (100)**	**699 (100)**	**832 (100)**

Table 4—Global shifts in different uroliths mineral submitted to the MUC (1984-1998) vs (1999-2012) compared.

	1984-1998	**1999-2012**	
Mineral variables	**Number/%**	**Number/%**	**Total Number/%**
Calcium carbonate	53 (39.8)	311 (44.5)	364 (43.8)
Calcium phosphate apatite	13 (9.8)	0 (0)	13 (1.6)
Calcium phosphate carbonate	1 (0.9)	4 (0.6)	5 (0.6)
Calcium oxalate dehydrate	0 (0)	6 (0.9)	6 (0.7)
Calcium oxalate monohydrate	0 (0)	1 (0.1)	1 (0.1)
Compound	4 (3)	42 (6 %)	46 (5.5)
Magnesium ammonium phosphate .6H2O	10 (7.5)	19 (2.7)	29 (3.5)
Matrix	0 (0)	4 (0.6)	4 (0.5)
Magnesium calcium phosphate apatite	12 (9.1)	29 (4.1)	41 (4.9)
Magnesium calcium phosphate carbonate	2 (1.5)	171 (24.5)	173 (20.8)
Mixed	9 (6.7)	24 (3.4)	33 (4)
Other	1 (0.7)	6 (0.9)	7 (0.8)
Silica	27 (20.3)	82 (11.7)	109 (13)
Xanthine	1 (0.7)	0 (0)	1 (0.1)
Total	**133 (100)**	**699 (100)**	**832 (100)**

Table 5—Global per cent and odds ratios distribution of different urolith minerals retrieved from goats: (1984-1998) submissions compared to (1999-2012)

Continent	CC	CPA	CPC	CO	COPD	MAP	MAT.	MCPA	MCPC	MIXED	OTHER	SILICA	XAN	%	No.
Asia															
1984-1998	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1999-2012	0.0	0.0	0.0	0.0	0.0	0.0	0.0	50	50	0.0	0.0	0.0	0.0	0.29	2
P value	NA	NA	NA	NA	NA	NA	NA	0.1528	0.1528	NA	NA	NA	NA		
Europe															
1984-1998	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1999-2012	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.89	6
P value	0.1796	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		
N. America															
1984-1998	39.8	9.8	0.8	0.0	3	7.5	0.0	9	0.2	6.8	0.8	20.3	0.8	100	133
1999-2012	43.6	0.0	0.1	1	6	2.7	0.6	4	24.3	3.4	0.9	11.7	00	98.8	691
OR	4.7	108.7	15.5	1.7	2.6	13.9	1.7	11.9	0.11	12.1	5.2	8.7	15.6		
P value	< 0.001	0.0012	0.0935	0.7385	0.1809	< 0.001	0.7382	< 0.001	0.1165	0.0003	0.2450	< 0.001	0.0935		
ALL CONTINENTS															
1984-1998	39.8	9.8	0.8	0.0	3	7.5	0.0	9	1.5	6.7	0.8	20.3	0.8	100	133
1999-2012	44.5	0.0	0.6	0.6	6	2.7	0.6	4	24.5	3.4	0.9	11.7	0.0	100	699
OR	4.6	108.7	5.2	1.7	2.6	13.9	1.7	11.7	0.43	12.1	5.2	8.7	15.6		
P value	< 0.001	0.0012	0.2417	0.5367	0.1756	< 0.001	0.7332	< 0.001	0.2422	0.0003	0.2417	< 0.001	0.0921		
GRAND TOTAL	**364**	**13**	**5**	**7**	**46**	**29**	**4**	**41**	**173**	**33**	**7**	**109**	**1**		**832**

CHAPTER 8

Epidemiological evaluation of calcium carbonate uroliths in sheep.

SUMMARY

This retrospective cross-sectional case study evaluated urolith submissions data from 40 sheep with $CaCO_3$ uroliths. Uroliths were submitted to the Minnesota Urolith Center (MUC) between January 1, 1981 and December 31, 2014. The objective of this study was to determine whether the frequency of occurrence of $CaCO_3$ uroliths in sheep varied with breed, age, sex, reproductive status, anatomic location within the urinary tract, season of uroliths collection, and geographic location; and to determine whether the rates of sheep $CaCO_3$ uroliths submitted to the MUC varied over time. $CaCO_3$ uroliths were common in Dorset, Suffolk and mixed sheep breeds. The Mean ± SD age of sheep was 3.6 ± 2.3years, median was 3.5 years and the age range (0.8 to 11 years). Three sheep breeds with highest proportions of $CaCO_3$ were Dorset (21%), Suffolk (17%) and mixed (14%). These 3 breeds comprised 15 of 32 (52%) sheep in which breeds were reported. Calcium carbonate uroliths submitted were primarily from the lower portions of the urinary tract, [37 of 39 (95%)]; and only two [(2 of 39 (5%)] submissions were uroliths from the upper urinary tract. Between 1981 and 1997, 28 ovine urolith samples were submitted to the MUC; 6 (21%) were $CaCO_3$ and 22(79%) were submissions of non- $CaCO_3$ urolith types. Between 1998 and 2014, 139 ovine urolith samples were submitted to the MUC; 34(25%) were CaCO3 and 105 (75%) were submissions of non- $CaCO_3$ urolith types. The proportion of $CaCO_3$ urolith submissions from sheep increased from 21% to 25% between the two periods. Knowledge of the predominant mineral type of uroliths along with insight into etiologic, demographic, and environmental risk and protective factors for urolithiasis may facilitate development of surveillance strategies that results in earlier

detection of uroliths in sheep. Modification of risk and protective factors may help to minimize urolith formation, and/ or urolith recurrence.

INTRODUCTION

Uroliths are macroscopic concretions that form within the urinary tract. They are primarily composed of minerals filtered by the kidneys, but can form from any substance that is sparingly soluble in urine. Calcium carbonate urolith is a common urolith reported in sheep.[4] Although urolithiasis has been recognized in sheep for about a half of a century, the pathophysiology of urolith formation in sheep is not fully understood.[19, 133-134]

As the frequency of detection of CaCO3 uroliths in sheep increases, knowledge of risk and protective factors for urolith formation is needed to develop effective diagnostic, therapeutic, and prevention strategies. The purpose of this study is to determine whether breed, age, sex, reproductive status, and geographic residence of urolith-formers and season of detection are most common in sheep that form $CaCO_3$ uroliths.

MATERIALS AND METHODS

Identification of cases —Medical records of the Minnesota Urolith Center[a] (MUC) were reviewed to identify sheep uroliths submitted by veterinarians or owners between January 1, 1981 and December 31, 2014. The mineral composition of the uroliths was determined by optical crystallography [42, 79] and, when necessary, by infrared spectroscopy. [42] Case animals were identified as sheep that had uroliths consisting of ≥ 70% $CaCO_3$. Animals with compound uroliths (i.e., those containing a nidus and outer layers ≥ 70% of different minerals) were excluded.

The following information was retrieved from urolith submission records: breed, age, sex, reproductive status (neutered or sexually intact), and state of residence of the case sheep; date of urolith submission, season of retrieval and anatomic location of the uroliths. Diet types were supplied for some of the sheep. Geographic regions[j] (southeast, southwest, mid-west, northeast, or west) were determined on the basis of state of residence according to classification established by the National Geographic education division.[c] Seasons of sample collection were classified as winter (December, January, February), spring (March, April, May), summer (June, July, August), and fall (September, October, November). The location of uroliths retrieved from the kidneys or ureters were classified as upper tract uroliths and uroliths retrieved from the bladder and the urethra were classified as lower tract uroliths. If uroliths were submitted more than once for the same animal, only the first submission was included in the analysis.

Statistical analysis— Risk factor is a variable associated with an increased occurrence of disease or infection. In this study risk is defined as the increased occurrence of $CaCO_3$ uroliths in sheep. Therefore, risk can be computed as follows:

$$\textbf{Risk} = \frac{\textbf{Number of sheep with CaCO}_3\textbf{ uroliths}}{\textbf{Number of sheep exposed to CaCO}_3\textbf{ uroliths.}}$$

As a result of the absence of controls in our study, this paper will be using proportion as the primary measure of risk of urolith frequency in sheep.

Standard statistical software [44, feck] was used to determine descriptive statistics (mean, median, and SDs) of breed, age, sex, reproductive status, and geographic location of sheep cases; season of urolith submission and anatomic location of the uroliths within the urinary tract. Since we were unable to obtain reliable controls we have included descriptive statistics in this section of the study. For analysis of age effect; < 1 year, 1 to < 3 years, 3 to < 5 years, 5 to < 7 years, 7 to < 9 years and 9 to > 11 years, sheep were divided into 6 age groups. To obtain more specific data on age effect, data for sheep < 5 years of age were also compared to sheep ≥ 5 years old. Relative frequencies were used to describe the breed, age, sex, reproductive status, and geographic location of sheep, season of urolith collection, and anatomic location of uroliths.

Prevalence of uroliths was expressed as relative frequencies of all the ovine uroliths submitted for analysis to the MUC. This computation was performed to determine whether the frequency of $CaCO_3$ uroliths retrieved from sheep submitted to the MUC increased or decreased over the study period. The 28-year study was grouped into 2 intervals ([1981 through 1997], and [1998 through 2014]) to determine whether the frequency of sheep with $CaCO_3$ changed over time.

RESULTS

Between January 1, 1981 and December 31, 2014, uroliths from 167 sheep resident in the USA were submitted to the MUC for analysis. Of these, 40 (24%) were $CaCO_3$ (**Table 1**). Thirty-nine uroliths were pure (100%) $CaCO_3$ and one urolith was (≥70%-99%).

Breed—Breed data were provided for 29 of 40 (72.5%) sheep with $CaCO_3$ uroliths (Table 3). The most common breeds with $CaCO_3$ uroliths were Dorset [6 of 29 (20.7%)], Suffolk [5 of 29 (17.2%)] and mixed breed [4 of 29 (13.8%)]. Breed data for 11 of 40 (27.5%) sheep were not provided at the time of urolith submission.

Age— Age data were provided for 37 of 40 (92.5%) sheep (Table 4). The Mean ± SD age of sheep with $CaCO_3$ uroliths was 3.4 ± 1.2 years (range = 0.8 to 11 years). Thirty-one of 37 (83.8%) sheep were < 5 years and 6 of 37 (16.2%) were ≥ 5 years of age. Sheep < 1 year of age comprised 2 of 37 (5.4%); 1 to < 3 years comprised 11 of 37 (29.7%); 3 to < 5 years comprised 18 of 37 (48.6%), and 5 to < 7 years comprised 3 of 37 (8.1%), 7 to < 9 years comprised 1 of 37 (2.7%), and 9 to 11 years comprised 2 of 37 (5.4%)**.** The ages for 3 of 40 (7.5%) sheep were not provided.

Sex and reproductive status—Of the 40 sheep, sex was recorded for 37 (92.5%; Table 4). Thirty-three of 37 (89.2%) were males, and 4 of 37 (10.8%) were females. The sex for 3 of 40 (7.5%) sheep was not provided. A higher proportion of sexually intact sheep [27 of 37 (73%)] had CaCO3 uroliths, compared to neutered sheep [(10 of 37 (27%)].

Seasonal distribution— Urolith submitted to the MUC were more in winter compared to other seasons (**Table 4**). Ten of 40 (25%) were submitted in fall, 8 of 40 (20%) in summer, 16 of 40 (40%) in winter, and 6 of 40 (15%) in spring.

Geographic distribution—Compared to other geographic regions, more submissions of $CaCO_3$ uroliths were received from the west and the mid-west regions of the United States (**Table 4**). Of the 40 CaCO3 urolith submissions, 9 of 40 (22.5%) were submitted from the west, 18 of 40 (45%) from the mid-west, 7 of 40 (17.5%) from the southwest, 3 of 40 (7.5%) from the northeast, and 3 of 40 (7.5%) from the southeast.

Anatomic location of uroliths—Locations from which the uroliths were collected were reported for 39 of 40 sheep (Table 5). Thirty-seven of 39 (95 %) sheep had uroliths obtained from the lower portions of the urinary tract. Uroliths from 2 sheep (5%) were obtained from the upper portions of the urinary tract (kidney or ureter). Fifteen (38 %) uroliths were retrieved from the bladder and 15 (38 %) from the urethra. Urolith submitted from both upper and lower portions of the urinary tract was 1 (2.5 %). Voided uroliths were 1(2.5%). Submission of uroliths from both upper and lower urinary tracts at the same time was uncommon. The anatomic location of 1 of 40 (2.5%) urolith was not recorded.

Method of uroliths retrieval—Of the 40 sheep with $CaCO_3$ uroliths submitted to the MUC; the retrieval method for 34 (85%) were not reported. Of the 6 urolith episodes for which the method of retrieval was recorded, 4 (66.7%) were by necropsy, and 2 (33.3%) were by surgery **(Table 7).** This may be as a result of urinary tract obstruction that subsequently ended up in surgery.

Urolith submission rate changes over time— Ovine $CaCO_3$ urolith submissions to the MUC progressively increased from 6 in the period of 1981 to 1997; to 34 in the period of 1998 to 2014. Calcium carbonate uroliths submission rates to the MUC increased from 21% between 1981 and 1997 to 25% between 1998 and 2014 compared to non- $CaCO_3$ urolith types retrieved from sheep during the same period with 79% and 75% respectively (**Table 2**).

DISCUSSION

The results of the present study supported our hypothesis that multiple factors such as breed [21% (6 of 29)], age [21% (18 of 37)], sex [89% (33 of 37)], reproductive status [72% (27 of 37)], season [40% (16 of 40)], and geographic location [45% (18 of 40)] of sheep with uroliths play a role in $CaCO_3$ urolith formation (**Table 4**). Our results are in agreement with reports by other investigators.[134-135] Calcium carbonate uroliths in sheep were typically round in shape. They were golden in color and ranged from 1 mm to >1 cm in diameter (**Figure 1**).

As theorized in urolith formation in other species; it is probable that when several risk factors occur together, their individual effects may be enhanced. Thus, each risk factor / or protective factors may play either a limited or a major role in the development or prevention of $CaCO_3$ uroliths. [77] The number of sheep with $CaCO_3$ uroliths in this study reflected an increased awareness that uroliths can cause urethral obstruction and increased use of diagnostic methods to detect uroliths such as survey portable radiography (survey radiography and abdominal ultrasonography). Submission rates may also have been associated with an increased awareness of the availability of complimentary urolith analysis that influenced submissions rates at the MUC.

The results of our study indicated that the frequency of Dorset, mixed and Suffolk sheep breeds with $CaCO_3$ uroliths were higher compared to other breeds. Familial predisposition for urolithiasis has been well documented for some mineral types.[80] For example, genetic mutations causing cystine urolithiasis have been identified in dogs [70] humans, and cats.[71]To the authors' knowledge a genetic predisposition for $CaCO_3$ uroliths in sheep has not been explored.

In our study, age was associated with development of $CaCO_3$ uroliths in sheep, with sheep between the ages of 3 and 5 having more $CaCO_3$ uroliths (78%) compared to other age groups (Table 4). Epidemiological data derived from studies [55-57] of dogs, cats, and humans have suggested that advancing age decreases the risk for urolthiasis. This is not surprising considering that the normal life span of sheep is between 10 and 12 years.
Our study has depicted that $CaCO_3$ uroliths in sheep is primarily observed in males compared to females. This is likely due to the anatomical and functional differences between male and female urinary tracts. This is likely related to the fact that males' urethras are sufficiently longer and narrower than those of females.[82]As urocystoliths pass into the distal end of the male urethra (urethral process); obstructive urolithiasis is a likely consequence. The S-shaped sigmoid flexure located just caudodorsal to the testes in male is another potential site for uroliths to lodge. It is likely that $CaCO_3$ uroliths are

not commonly detected in females because the wider and shorter urethra may allow uroliths to be passed in the urine stream before they can cause clinical signs.

A higher proportion of $CaCO_3$ uroliths were from sexually intact (73%) sheep than neutered (27%) sheep. This observation differed from what we have observed in other uroliths retrieved from cats, dogs, and ferrets, where our observation suggested that neutering may be a risk factor for urolithiasis. This observation may be as a result of the very few CaCO3 uroliths from sheep that was involved in this study (40 cases). It is probable that male sheep are kept primarily for breeding purposes and therefore are not routinely subjected to neutering as in ferrets, goats and horses. Another possibility is that sheep are presently not routinely used as pets compared to goats and therefore are less likely to be neutered. Neutering in pet animals are used mainly for over population control measure. These differences may be due to the few sheep that were involved in this study. There is need for studies that will involve control sheep to be used to determine the risk and protective factors associated with $CaCO_3$ uroliths in sheep.

Thirty-seven of 39 (95%) sheep in this study had $CaCO_3$ uroliths retrieved from the lower portions of the urinary tract (Table 6). The predominant location of uroliths in the lower portions of the urinary tract of other domestic animals has been described.[84]In our experience, the retrieval of uroliths from the upper urinary tract is likely underreported, because survey radiography and ultrasonography are often times omitted during initial evaluation of farm animals with clinical signs of urolithiasis. Clinical signs caused by uroliths in the lower urinary tract (increased voiding frequency, straining to void, and dribbling urine) are more likely to be observed than are clinical signs caused by uroliths located in the upper urinary tract (polyuria and hematuria without obvious signs of pain, which may go unrecognized). In the sheep of the present report, uroliths were equally retrieved from the urethra and the bladder with 15 of 39 (38%) each compared to other locations. These localizations are important when considering dietary modification for dissolution of uroliths. It has been recommended that attempts to dissolve urethral uroliths by dietary modification be avoided.[84] Uroliths lodged in the ureters or urethra cannot be dissolved by medical protocols because urolith dissolution requires sustained contact with urine that has been modified and therefore is undersaturated with lithogenic mineral.[84]

In our study, $CaCO_3$ uroliths were detected more in sheep residing in the mid-west, than did sheep from the other regions. These observations indicate that geographic location may be associated with $CaCO_3$ urolithiasis. The mid-west region has the highest proportion of sheep with $CaCO_3$ in this study. This observation may constitute a form of bias because of the proximity of the MUC laboratory to the mid-west region. Geographic prevalence of urolithiasis has been reported in dogs and humans.[85-86]Our study was not designed to determine the reason for the variations among geographic locations. Further research is needed to assess the factors that contribute to such differences.

Calcium carbonate uroliths were more likely to be retrieved from sheep in winter than in other seasons. The association between season and presence of urolithiasis is in agreement with findings of other investigators, who reported that detection of urolithiasis in cats, sheep, and humans varies with season.[87-89]This may be due to reduced water intake during the winter season that could have led to increased urine concentration. Increased urine concentration is a risk factor for urolith formation. We did not investigate possible associations between $CaCO_3$ urolithiasis and these factors.

Diet has been incriminated as important risk factor for $CaCO_3$ urolith formation in sheep.[90] In our study; we encountered a lack of detailed information regarding diet composition and variability in the reported diets fed to case sheep. Lack of consistency in response to our requests for dietary history prevented evaluation of dietary components as a factor in the frequency of occurrence for this disease. To our knowledge, controlled, blinded clinical studies have not been performed to evaluate associations between most dietary risk factors and uroliths formation in sheep. In our experience in other species it is likely that diet has a role to play in the pathogenesis of $CaCO_3$ urolithiasis. Most sheep cases for which diet was reported primarily received grass, and alfalfa hay 14 of 22 (64 %;) compared to other diets. Alfalfa and grass hay have a high calcium-to-magnesium concentration ratio and low phosphorus concentration and are also rich in oxalate. Further prospective studies are needed to define the role played by each of these factors singly and in combination as they relate to $CaCO_3$ urolithiasis in sheep.

Healthy sheep normally excrete urine that is alkaline (pH range, 7.5 to 8.5). Goats, horses, rabbits, and guinea pigs also normally excrete alkaline urine. Production of alkaline urine is shared characteristics among these herbivorous species. Ingestion of plants typically (e.g., grass and alfalfa) are associated with alkaline urine production.[91] Although, not investigated in the present study, it is probable that alkaline urine pH is a risk factor for $CaCO_3$ urolithiasis in sheep, and this should be investigated in future studies.

In our study the primary method of $CaCO_3$ uroliths retrieval in sheep were by necropsy [4 of 6 (66.7%)] and surgery [2 of 6 (33.3)] **(Table 7).**

Conclusion

Calcium carbonate comprised 24% ovine urolith submissions to the MUC. The 3 sheep breeds with highest proportions of $CaCO_3$ were Dorset (21%), Suffolk (17%) and mixed (14%). The Mean ± SD age of sheep with $CaCO_3$ uroliths was 3.4 ± 1.2 years (range = 0.8 to 11 years). The proportion of $CaCO_3$ were more in males and sheep 3 to < 5years of age. Calcium carbonate uroliths submitted were primarily from the lower urinary tract of sheep than from the upper urinary tract. The proportion of $CaCO_3$ urolith submissions from sheep increased during the study. Knowledge of the frequency of the predominant mineral types of uroliths in sheep may facilitate development of surveillance strategies that result in earlier detection of uroliths. Modification of risk factors may help to minimize urolith formation, and/ or minimize recurrences.

Biases and limitations Since our data was obtained from sheep urolith submissions to the MUC, it was not a complete representative of all urolith submissions from all breed, age, sex, reproductive status, season and geographic location of all sheep populations. Urethras in male sheep are substantially longer and narrower than in females. Also the males have urethral process and sigmoid flexure which are absent in females. All these features have contributed to more clinical manifestations of urethral obstruction in males compared to females and therefore may have influenced their selection and submission as cases as a bias selection.

Since the MUC uroliths analyses are performed at no cost to veterinarians this may have influenced the rate of submissions to the MUC. Also because the sample size of sheep involved in this study was not large we were unable to apply the conclusions derived from the findings of our study to the whole sheep populations.

Uroliths obtained from sheep in this study were retrieved mainly from the lower portions of the urinary tract. Clinical signs are more likely to be observed when uroliths are located in the lower portions of the urinary tract than from the upper portions of the urinary tract. This may have constituted a form of bias in this study leading to higher number of lower urinary tract uroliths being observed in this study compared to the upper urinary tract locations.

Furthermore, sheep in the present study most likely had clinical signs. Our study may not have included asymptomatic animals, sheep that voided uroliths uneventfully, or sheep whose uroliths were not collected and submitted to the MUC for analysis. Sheep that died as a result of urinary tract obstruction and were not necropsied or sheep from geographic areas where other urolith analysis laboratories may have solicited submission were also not represented. As a result of these limitations, this study may not have fully represented the population of all sheep with $CaCO_3$ uroliths and therefore may have constituted a form of bias.

Although information derived from extensive urolith data compiled by the MUC provided a unique resource for retrospective study, but because we have limited access to sheep with uroliths, we have directed most of our efforts toward the evaluation of the epidemiological features of this disorder. Knowledge of the epidemiology of urolithiasis is essential for constructing effective experimental designs and choosing appropriate cases and controls for conducting clinical trials with meaningful results that may facilitate development of surveillance strategies that result in earlier detection of uroliths in sheep.

Table 1—Mineral composition of 167 uroliths retrieved from sheep resident in the USA and submitted to the Minnesota Urolith Center between 1981and 2014

Variable	**Number of uroliths**	**%**
Calcium carbonate	40	23.9
Calcium phosphate apatite	12	7.1
Calcium phosphate carbonate	2	1.1
Compound	7	4.3
Struvite	23	13.8
Magnesium calcium phosphate apatite	9	5.4
Magnesium calcium phosphate carbonate	42	25.1
MISC Materials	4	2.4
Mixed	10	5.9
Silica	18	10.8
Total	**167**	**100**

Table 2—Breed distribution for 40 sheep with $CaCO_3$ uroliths resident in the USA and submitted to the MUC between 1981 and 2014

Breeds	Number	Per Cent
Barbados	2	5
Columbia	2	5
Dorset	6	15
Hampshire	2	5
karakul	2	5
Merino	2	5
Mixed	4	10
Navajo-Churro	1	2.5
Rambouilette	2	5
Shetland	1	2.5
Suffolk	5	12.5
Unknown	11	27.5
TOTAL	**40**	**100**

Table 3—Summary data for 40 sheep with $CaCO_3$ uroliths resident in the USA and submitted to the MUC between 1981 and 2014 indicating age, sex, and reproductive status, season and geographical location of stone-formers.

Variables	**Description**	**Number**	**Per cent**
Age	< 1 year	2	5
	1 – < 3 years	11	27.5
	3 - < 5 years	18	45
	5 - < 7 years	3	7.5
	7 - < 9 years	1	2.5
	9 - > 11 years	2	5
	Unknown	3	7.5
Total		**40**	**100**
Age category	< 5 years	31	77.5
	≥ 5 years	6	15
	Unknown	3	7.5
Total		**40**	**100**
Sex			
	Male	33	82.5
	Female	4	10
	Unknown	3	7.5
		40	100
	Neutered	10	25
	Sexually intact	27	67.5
	Unknown	3	7.5
Total		**40**	**100**
Season			
	Fall	10	25
	Spring	6	15
	Summer	8	20
	Winter	16	40
Total		**40**	**100**
Geographic location			
	Midwest	18	45
	Northeast	3	7.4
	Southwest	7	17.5
	Southwest	3	7.5
	West	9	22.5
Total		**40**	**100**

Table 4—Anatomic locations for 40 $CaCO_3$ uroliths retrieved from sheep resident in the USA and submitted to the MUC between 1981 and 2014

Anatomic location	Number	Per cent
Lower tract	37	92.5
Upper tract	2	5
Unknown	1	2.5
Total	**40**	**100**
Bladder	15	37.5
Bladder/urethra	6	15
Kidney	1	2.5
Kidney/ureter	1	2.5
Urethra	15	37.5
Void	1	2.5
Unknown	1	2.5
TOTAL	**40**	**100**

Table 5—Different type of diets used to feed 40 sheep with $CaCO_3$ uroliths resident in the USA and submitted to the MUC between 1981 and 2014

Diet	**Number**	**Per cent**
Grass/alfalfa hay/pasture/Timothy	14	35
Sweet feed	1	2.5
Linseed/corn/oat/barley	1	2.5
Pellet	2	5
Unknown	22	55
Total	**40**	**100**

Table 6—Methods of retrieval for 40 $CaCO_3$ uroliths from sheep resident in the USA and submitted to the MUC between 1981 and 2014

Method of retrieval	Number	Per cent
Necropsy	4	10
Surgical	2	5
Void	1	2.5
NA	33	82.5
Total	**40**	**100**

Table 7—$CaCO_3$ uroliths compared to non- $CaCO_3$ uroliths retrieved from sheep residence in the USA and submitted to the Minnesota Urolith Center between intervals of (1981-1997) and (1998-2014).

Stone Type	**Year of submission**			
	1981-1997		1998-2014	
	No.	%	No.	%
Calcium carbonate	6	21.4	34	24.5
Calcium phosphate apatite	9	32.1	3	2.1
Compound	1	3.6	6	4.3
MAP	5	17.9	18	12.9
Mixed	2	7.1	8	5.8
Silica	5	17.9	13	9.4
Calcium phosphate carbonate	-	-	2	1.4
Magnesium calcium phosphate apatite	-	-	9	6.4
Magnesium calcium phosphate carbonate	-	-	42	30.2
MISC Materials	-	-	4	2.9
Total	**28**	**100**	**139**	**100**

Figure 1—Photograph of calcium carbonate uroliths from 5-year-old intact male, mixed sheep. Measurements are shown in centimeters.

CHAPTER 9

Epidemiological evaluations of magnesium calcium phosphate carbonate (MCPC) uroliths in sheep.

SUMMARY

This cross-sectional retrospective case study evaluated urolith submissions data of 42 sheep with magnesium calcium phosphate carbonate (MCPC) uroliths. Uroliths were submitted to the Minnesota Urolith Center (MUC) between January 1, 1981 and December 31, 2014. The objective of this study was to determine whether the frequency of MCPC uroliths in sheep varied with breed, age, sex, reproductive status, anatomical location within the urinary tract, season of urolith collection, and geographical location; and to determine whether the frequency rates of sheep MCPC uroliths submitted to the MUC varied with time.

The Mean ± SD age of sheep with MCPC uroliths was 1.6 ± 1.2 years (range: 0.2 to 9 years). We hypothesize that sheep of a particular breed, age, sex and reproductive status, in certain seasons and geographic locations are associated with increased frequency for MCPC urolith formation. Suffolk sheep had the highest proportion of MCPC uroliths in this study and comprised 11 of 31 (35%). The breeds for 11 sheep were not reported. Magnesium calcium phosphate carbonate uroliths were more commonly seen in sheep < 1year of age [23 of 38 (61%); age unknown for 4 sheep].

For all sheep sex was reported for 41 of 42 (98%) at the time of urolith submission and unknown for 1 (1=unknown). There were no females among sheep for which sex was reported at the time of submission. Of the 41 of 42 sheep which had information regarding neuter status; 15 of 41 (37%) were neutered, while 26 of 41 (63%) were sexually intact. The frequency of MCPC was more among sexually intact sheep

compared to neutered group. Magnesium calcium phosphate carbonate uroliths in sheep were more commonly collected in spring and summer than in other seasons.

All MCPC uroliths retrieved from sheep and submitted to the MUC during the study period were retrieved from the lower portions of urinary tract. No MCPC were confirmed in sheep between 1981 and 1997. Between 1998 and 2014 MCPC comprised 30% (42 of 139) while other minerals comprised 70% (97 of 139). Knowledge of the frequency of MCPC uroliths along with insight into etiologic, demographic, and environmental factors for urolithiasis may facilitate development of surveillance strategies that result in earlier detection of uroliths in sheep. Modification of these factors may help to minimize urolith formation, dissolve existing uroliths, and/ or minimize urolith recurrence.

INTRODUCTION

Uroliths are macroscopic concretions that form within the urinary tract. They are primarily composed of minerals filtered by the kidneys, but can form from any substance that is sparingly soluble in urine. Although urolithiasis has been recognized in sheep for about a half of a century, the pathophysiology of urolith formation in sheep is not fully understood.[19, 134]

As the frequency of detection of MCPC uroliths in sheep increases, knowledge of the associated risk and protective factors for urolith formation is needed to develop effective diagnostic, therapeutic and prevention strategies. The purpose of this study is to determine which breed, age, sex and reproductive status, anatomic location within the urinary tract, geographic residence of urolith-formers and season of collection are most common in sheep that form MCPC uroliths.

MATERIALS AND METHODS

Identification of cases—Medical records of the MUC[a] were reviewed to identify sheep with urolith submissions by veterinarians or owners between January 1, 1981 and December 31, 2014. The mineral composition of the uroliths was determined by optical crystallography [42, 79] and, when necessary, by infrared spectroscopy.[42] Case animals were identified as sheep that had uroliths consisting of $\geq$ 70% MCPC. Records of sheep with compound uroliths (i.e., those containing a nidus and outer layers $\geq$ 70% of different mineral types) were excluded.

The following information was retrieved from urolith submission records: breed, age, sex, reproductive status (neutered or sexually intact), and state of residence of the sheep urolith-formers; date of urolith submission; and anatomic location of the urolith.

Diet type was supplied for some of the sheep. Geographic regions (southeast, southwest, mid-west, northeast, or west) were determined on the basis of state of residence of the sheep according to the classification established by the National Geographic education division[j]. Seasons[h] of sample collection were classified as winter (December, January, February), spring (March, April, May), summer (June, July, August), and fall (September, October, November). The location of uroliths retrieved from the kidneys or ureters were classified as upper tract uroliths and uroliths retrieved from the bladder and urethra were classified as lower tract uroliths. If uroliths were submitted more than once for the same animal, only the first submission was included in the analysis.

Statistical analysis—Standard statistical software [44, d, e] was used to determine descriptive statistics (mean, median, and SDs) of breed, age, sex, reproductive status, geographic location of sheep at the time of urolith retrieval, season of urolith submission, and anatomic location of the uroliths within the urinary tract. Since we were unable to obtain reliable controls we have included descriptive statistics in this section of the study. For analysis of age effects, < 1 year, 1 to < 3 years, 3 to < 5 years, 5 to < 7 years, 7 to < 9 years, and 9 to > 11 years, sheep were divided into 6 age groups. To obtain more specific data on age effect, data for sheep < 5 years of age were also compared with those of ≥ 5 years old. Relative frequencies were used to describe the breed, age, sex, reproductive status, and geographic location of sheep, season of urolith collection, and anatomic location of uroliths.

Prevalence of MCPC uroliths was expressed as the relative frequency of all uroliths submitted for analysis to the MUC from sheep residing in the USA. This computation was performed to determine whether the frequency of occurrence of MCPC uroliths changed over time. The 28-year study was grouped into 2 intervals (1981 through 1997) and (1998 through 2014) to determine whether the frequency of occurrence of MCPC changed over time.

Risk factor is a variable associated with an increased occurrence of disease or infection. In this study risk is defined as the increased occurrence of MCPC uroliths in sheep. Therefore, risk can be computed as follows:

$$\textbf{Risk} = \frac{\textbf{Number of sheep with MCPC uroliths}}{\textbf{Number of sheep exposed to MCPC uroliths.}}$$

As a result of the absence of controls in our study, this paper will be using proportion as the primary measure of risk of urolith frequency in sheep.

RESULTS

Between January 1, 1981 and December 31, 2014, uroliths from 167 sheep resident in the USA were submitted to the MUC for analysis. Of these, MCPC comprised 42 (25.1%) (**Table 1**). Forty uroliths were pure (100%) MCPC and 2 were ≥ 70-99%. Magnesium calcium phosphate carbonate is the most common urolith in sheep. Ovine MCPC urolith submissions to the MUC progressively increased from zero in the period of 1981 to 1997; to 42 in the period of 1998 to 2014. Magnesium calcium phosphate carbonate uroliths retrieved from sheep were typically irregular in shape. They were brown in color and range from 1 mm to > 1cm in diameter (**Figure 1**).

Breed—Breed data were provided for 31 of 42, (74%) urolith submissions. The most common breeds with MCPC uroliths was Suffolk [11 of 31 (35.5%); **Table 2**].

Age—Age data were provided for 38 of 42 (90%) sheep at the time of urolith submission (**Table 3**). The Mean ± SD age of sheep with MCPC uroliths was 1.6 ± 1.2 years (range:

0.2 to 9 years). The proportion of sheep with MCPC was highest in animals < 1 year of age [23 of 38 (60.5%)]. Thirty-three of 38 (86.8%) sheep were < 5 years and 5 of 38 (13.2%) sheep were ≥ 5 years of age. Sheep 1 to < 3 years comprised 9 of 38 (23.7%); 3 to < 5 years comprised 1 of 38 (2.6%); 5 to < 7 years comprised 3 of 38 (7.9%), 7 to < 9 years comprised 1 of 38 (2.6%) and 9 to > 11 years comprised 1 of 38 [(2.6%); **Table 4**]. The ages for 4 of 42 (9.5%) sheep were not provided.

Sex and reproductive status—Of the 42 sheep, sex was recorded for 41 (97.6% **Table 3**). All the 41 sheep for which sex was recorded were males. Fifteen (36.6%) were neutered and 26 (63.4%) were sexually intact. A higher proportion of sexually intact sheep had MCPC uroliths, compared to neutered (**Table 3**).

Seasonal distribution—Urolith submissions to the MUC were more in spring compared to other seasons (**Table 3**). Seventeen of 42 (40.5%) were submitted in spring, 11 of 42 (26.2%) in summer, 8 of 42 in winter, and 6 of the 42(14.3%) were submitted in fall.

Geographic distribution—Compared to other geographic regions, more submissions of MCPC uroliths were submitted from the mid-west of the USA**.** Of the 42 MCPC urolith submission, 3 of 42 (7.1%) were submitted from the northeast, 4 of 42 (9.5%) from the southeast, 5 of 42 (11.9%) from the southwest, 8 of 42 (19.1%) the west, and 22 of 42 (52.4%) from the mid-west (**Table 3**).

Anatomic location of uroliths—Locations from which the uroliths were retrieved were reported for 40 of 42 (95.2%) sheep; **Table 4**). All the 40 MCPC uroliths were retrieved from the lower portions of the urinary tract (urinary bladder, urethra, voided uroliths, or some combination of these). Uroliths retrieved from the bladder only was 20 (50%) and from the urethra only was 13 (32.5%; **Table 4**).

Diet—In 13 of 42 (31%) the diets used were reported. In 29 of 42 (69%) the diets used were not reported (**Table 5**).

Method of uroliths retrieval—Of the 42 MCPC uroliths submitted to the MUC, the retrieval methods for 25 (59.5%) were not documented. Of the 17 urolith episodes for which the method of retrieval was recorded, 10 (58.8%) were by necropsy and 7 (41.2%) were through surgery. This may be as a result of urinary obstruction that subsequently ended in necropsy.

History of previous uroliths—Of the 42 sheep with MCPC uroliths submitted prior history was not reported for 29 (69%) uroliths. For those that did 12 of 13 (92.3%) had no previous history of urolith, and 1 of 13 (7.7%) had history of previous uroliths.

Change of MCPC urolith in sheep over time—Magnesium calcium phosphate carbonate uroliths retrieved from sheep residing in the USA increased from 0 of 28 (0%) between 1981 and 1997 to 42 of 139 (30.2%) between 1998 and 2014 [**Table 6**]. On the other hand, the other minerals retrieved from sheep during the same interval decreased from 28 of 28 [(100%) to 97 of 139 (69.8%); **Table 6)**

DISCUSSION

The result of the present study supported our hypothesis that breed [11 of 31 (35%)], age [23 of 38 (61 %)], sex [was all males for those reported], and reproductive status [26 of 41 (63%)], likely play a role in the frequency of occurrences of MCPC urolith in sheep. Our reports were in agreement in breed, age, and sex, but not in reproductive status as reported by other investigators.[133-134]

As theorized in urolith formation in other species; it is probable that when several risk and protective factors occur together, their individual effects may be enhanced. Thus, each frequency factor or protective factor may play either a limited or a major role in the development or prevention of MCPC uroliths.[77]

The number of sheep with MCPC uroliths in this study may have reflected an increased awareness in owners that uroliths can cause urethral obstruction or increased availability of diagnostic methods to detect uroliths such as survey radiography and abdominal ultrasonography. Submission rates may also have been associated with an increased awareness of the availability of complimentary urolith analysis at the MUC. This was buttressed by the fact that MCPC was zero per cent between 1981 and 1997; and increased to 30.2% between 1998 and 2014.

The results of our study indicated that the Suffolk breed of sheep had a highest proportion of MCPC [11 of 31 (35.5%)]. Familial predisposition for urolithiasis has been well documented in various species for some mineral types.[80] For example, genetic mutations causing cystine urolithiasis have been identified in dogs [70] humans, and cats.[71]To the authors' knowledge a genetic predisposition for MCPC uroliths in sheep has not been explored. Prospective and retrospective studies involving control sheep are necessary to further determine the risk factors for MCPC uroliths in this species.

In our study, age was highly associated with detection of MCPC in sheep. Sheep < 1 year-old having the highest frequency compared to other age groups (**Table 3**). This is not surprising because MCPC urolithiasis is a disease of young animals and the normal lifespan of a sheep is 10 to 12 years. Bacterial urinary tract infections are common in female and dogs of all ages and in our study all were males. As a result, it is hard to draw any conclusion from this observation. There have been no reports of urease-producing microbes that are involved in infection-induced MCPC urolithiasis in sheep to the authors' knowledge.

Our study has depicted that MCPC uroliths are primarily observed in males compared to females. This is likely related to the fact that males' urethras are substantially longer and narrower than those of females.[82]As urocystoliths pass through the distal end of the male urethra (urethral process); obstructive urolithiasis is a likely consequence. The S-shaped sigmoid flexure located just caudodorsal to the testes is another potential site for uroliths to lodge. It is probable that MCPC uroliths are not commonly detected in female sheep because the substantially wider and shorter urethra allows uroliths to be passed in the urine stream before they can cause clinical signs**.**

A higher proportion of MCPC uroliths were from sexually intact (63%) sheep than neutered (37%). This observation differed from what we have observed in other uroliths retrieved from other animals where our observation suggested that neutering may increase the frequencies of urolithiasis.[24] Our observation may be due to very few MCPC uroliths retrieved from sheep that were involved in this study (42 cases). It also may have arisen from the preferences for keeping sexually intact sheep for breeding purposes as against neutering them. Equally true is the fact that sheep are not routinely used as pets in the US compared to goats and as a result they are less likely to be neutered when compared to pet goats. Neutering are performed in pet animals so as to control population explosion. Because the ages at which sheep in this study were neutered was not provided, we were unable to determine whether there was a significant association between urolithiasis in sheep and early neutering. These differences may be due to the few sheep that were involved in this study. There is need for studies that will involve control sheep to be performed so as to determine the risk and protective factors for MCPC uroliths in sheep.

Forty of 42 (100%) sheep cases in this study had MCPC uroliths retrieved from the lower portions of the urinary tract (**Tables 4**). None of the uroliths was retrieved from the upper portions of the urinary tract. The anatomic locations for 2 uroliths were not reported. The predominant location of uroliths in the lower portions of the urinary tract of other domestic animals has been described.[84]In our experience in dogs, cats, ferrets and other species, the development of uroliths in the upper portions of the urinary tract is likely underreported, because survey radiography and ultrasonography are often times omitted during initial evaluation of animals with clinical signs of urolithiasis. Clinical signs caused by uroliths in the lower portions of the urinary tract (increased voiding frequency, straining to void, and dribbling urine) are more likely to be observed than are clinical signs caused by uroliths located in the upper portions of the urinary tract (polyuria and hematuria without obvious signs of pain, which may go unrecognized).

In sheep of the present report, uroliths were less commonly retrieved from the urethra only [13 of 40 (32.5%)\ compared to the bladder only [20 of 40 (50%)]. These localizations are important when considering dietary modification for dissolution of uroliths. It has been recommended that attempts to dissolve urethral uroliths by dietary modification be avoided.[84] Uroliths lodged in the ureters or urethra cannot be dissolved by medical protocols because urolith dissolution requires sustained contact with urine that has been modified and therefore is undersaturated with lithogenic mineral.[84] Because the proportion of bladder uroliths retrieved from sheep were more than those retrieved from urethra, medical dissolution of uroliths are very feasible in sheep.

In our study, MCPC uroliths were detected more in sheep residing in the mid-west compared to those residing in the other regions of the USA. These observations indicate that geographic location may be a risk factor for urolithiasis. Geographic prevalence of urolithiasis has been reported in dogs and humans.[85-86]Our study was not designed to determine the reason for the variations among geographic locations. It is also probable that the location of MUC laboratory in the mid-west region of the USA may have been responsible for the increase in number of uroliths received from this region and it may have constituted a form of bias in the study. Further research is needed to assess the factors that contribute to such differences.

Magnesium calcium phosphate carbonate uroliths were more likely to be retrieved from sheep during spring than in other seasons. The association between season and frequency of urolithiasis is in agreement with findings of other investigators, who have reported that detection of urolithiasis in cats, sheep, and humans varied with season.[86-90]

Magnesium calcium phosphate carbonate uroliths retrieved from sheep residing in the USA increased from 0 of 28 (0%) between 1981 and 1997 to 42 of 139 (30.2%) between 1998 and 2014 [**Table 5**]. On the other hand, the other minerals retrieved from sheep during the same interval decreased from 28 of 28 [(100%) to 97 of 139 (69.8%); **Table 5)**. This may most likely be due to the increased awareness of this mineral type. Prior to 1998, uroliths that were composed of MCPC may have been erroneously reported as silica (oral communication). It is also possible that this may have been due to reciprocity between MCPC uroliths and other minerals retrieved from sheep during the same period because while the former increased the later decreased. However, further study is needed to substantiate this observation with more sheep with MCPC uroliths.

Diet has been incriminated as important risk factor in the frequency of occurrences of urolith in sheep.[91]In our study; we encountered a lack of detailed information regarding diet composition and variability in the reported diets fed to sheep. Lack of consistency in response to our requests for dietary history prevented evaluation of the association of dietary components and MCPC urolithiasis. To our knowledge, controlled, blinded clinical studies have not been performed to evaluate associations between most dietary factors and urolith formation in sheep. In our experience, in cats, and dogs, it is likely that diet has a role to play in the pathogenesis of MCPC urolith formation in sheep. Further prospective studies are needed to define the role played by each of these factors singly and in combination as they relate to MCPC urolith formation in sheep.

Healthy sheep normally excrete urine that is alkaline (pH range, 7.5 to 8.5). Goats, horses, rabbits, and guinea pigs also normally excrete alkaline urine. Production of alkaline urine is shared characteristics among these herbivorous species. Ingestion of plants typically (e.g., grass hay, alfalfa hay and pasture) are associated with alkaline urine production.[90-91] Although, not investigated in the present study, it is probable that alkaline urine pH is a risk factor for MCPC urolithiasis in sheep, and this should be investigated in future studies.

In our study the primary method of MCPC uroliths retrieval in sheep were by necropsy [10 of 18(55%)] and [7 of 18 (39%)] were by surgery (**Table 6**). This may be a reflection of “herd health’’ mentality, or may be associated with “prey’’ status of sheep. Sheep tend to hide their weakness to avoid predation.

Conclusion

The Mean ± SD age of sheep with MCPC uroliths was 1.6 ± 1.2 years (range: 0.2 to 9 years). Suffolk sheep had the highest proportion of MCPC uroliths in this study and comprised 11 of 31 (35%). Magnesium calcium phosphate carbonate uroliths were more common in sheep < 1year of age [23 of 38 (61%). The frequency of MCPC was more among sexually intact sheep compared to neutered group. Magnesium calcium phosphate carbonate uroliths in sheep were more commonly received from the Mid-west than from any other region. Magnesium calcium phosphate carbonate uroliths in sheep were more commonly collected in spring than in other seasons.

All MCPC uroliths retrieved from sheep and submitted to the MUC during the study period were retrieved from the lower portions of urinary tract. No MCPC were confirmed in sheep between 1981 and 1997. Between 1998 and 2014 MCPC comprised 30% (42 of 139) while other minerals comprised 70% (97 of 139). Knowledge of the frequency of MCPC uroliths along with insight into etiologic, demographic, and environmental factors for urolithiasis may facilitate development of surveillance strategies that result in earlier detection of uroliths in sheep. Modification of these factors may help to minimize urolith formation, dissolve existing uroliths, and/ or minimize urolith recurrence.

Biases and limitations

Since our data was obtained from sheep urolith submissions to the MUC, it was not a complete representative of all urolith submissions from sheep of all breed, age, sex, reproductive status, season and geographic location.

Urethras of male sheep are substantially longer and narrower than that of females. Also the males have urethral process and sigmoid flexure which are absent in females. All these anatomic features may have contributed to more clinical manifestations of urethral obstruction in male sheep compared to females and may have therefore influenced their selection and subsequent submission as cases.

Since the MUC uroliths analyses are performed at no cost to veterinarians and owners this may have influenced the rate of urolith submissions to the center. Also because the sample size of sheep in this study was not very large and absence of reliable control sheep, we were unable to apply the conclusions derived from the findings of our study to the whole sheep populations.

Uroliths obtained from sheep in this study were retrieved mainly from the lower portions of the urinary tract. Clinical signs are more likely to be observed when uroliths are located in the lower portions of the urinary tract than from the upper portions. This may have constituted a form of bias in this study leading to higher number of lower urinary tract uroliths being observed compared to the upper portions of the urinary tract location.

Furthermore this study may have included only clinical cases, and may not have included asymptomatic cases (subclinical) or cases that may have been euthanized without a subsequent necropsy, sheep from geographical areas where other urolith analysis laboratories may have solicited submission from their surroundings, sheep that have voided uroliths uneventfully, or sheep whose uroliths were not collected and submitted to the MUC for analysis ; as a result this may have limited the use of results from our study to fully represent the population of every sheep with MCPC uroliths and therefore may have constituted a form of bias.

Table 1--Mineral composition of 167 uroliths retrieved from sheep resident in the USA and submitted to the Minnesota Urolith Center between 1981and 2014.

Variable	**Number of uroliths**	**%**
Magnesium calcium phosphate carbonate	42	25.1
Calcium phosphate apatite	12	7.1
Calcium phosphate carbonate	2	1.1
Compound	7	4.3
Magnesium ammonium phosphate 6H2O	23	13.8
Magnesium calcium phosphate apatite	9	5.4
Calcium carbonate	40	23.9
MISC Materials	4	2.4
Mixed	10	5.9
Silica	18	10.8
Total	**167**	**100**

Table 2—Breed distribution for 42 sheep with MCPC uroliths resident in the USA and submitted to the MUC between 1981 and 2014.

Breeds	Number	Per Cent
Dorset	2	4.7
Hampshire	6	14.3
Jacobs Sheep	1	2.4
Mixed	3	7.1
Rambouilette	6	14.3
Shetland	1	2.4
Suffolk	11	26.2
Western	1	2.4
Unknown	11	26.2
TOTAL	**42**	**100**

Table 3 —Summary data for 42 sheep with MCPC uroliths resident in the USA and submitted to the MUC between 1981 and 2014 indicating age, sex, reproductive status and season.

Variables	Description	Number	Per cent
Age	< 1 year	23	54.8
	1 – < 3 years	9	21.4
	3 - < 5 years	1	2.4
	5 - < 7 years	3	7.1
	7 - < 9 years	1	2.4
	9 - > 11 years	1	2.4
	Unknown	4	9.5
Total		**42**	**100**
Sex			
	Males	41	98
	Females	0	0
	Unknown	1	2
Total		**42**	**100**
Reproductive status			
	Neutered	15	35.7
	Sexually intact	26	61.9
	Unknown	1	2.4
Total		**42**	**100**
Season			
	Fall	6	14.3
	Spring	17	40.5
	Summer	11	26.2
	Winter	8	19
Total		**42**	**100**
Geographical location			
	Midwest	22	52.4
	Northeast	3	7.1
	Southeast	4	9.5
	Southwest	5	11.9
	West	8	19
Total		**42**	**100**

Table 4—Anatomic locations for 42 sheep with MCPC uroliths resident in the USA and submitted to the MUC between 1981 and 2014.

Anatomic location	**Number**	**Per cent**
Lower tract	40	95.2
Upper tract	0	0
Unknown	2	4.8
Total	**42**	**100**
Bladder	20	47.6
Bladder/urethra	6	14.3
Urethra	13	30.9
Voided	1	2.4
Unknown	2	4.8
TOTAL	**42**	**100**

Table 5—Methods of retrieval for 42 MCPC uroliths retrieved from sheep resident in the USA and submitted to the MUC between 1981 and 2014.

Method of retrieval	Number	Per cent
Necropsy	10	23.8
Surgical	7	16.7
Void	1	2.4
Unknown	24	57.1
Total	**42**	**100**

Table 6—MCPC **uroliths compared to other minerals retrieved from sheep residence in the USA and submitted to the Minnesota Urolith Center between intervals of (1981-1997) and (1998-2014).**

Stone Type	**Year of submission**			
	1981-1997		1998-2014	
	No.	%	No.	%
Magnesium calcium phosphate carbonate	-	-	42	30.2
Calcium phosphate apatite	9	32.1	3	2.1
Compound	1	3.6	6	4.3
MAP	5	17.9	18	12.9
Mixed	2	7.1	8	5.8
Silica	5	17.9	13	9.4
Calcium phosphate carbonate	-	-	2	1.4
Magnesium calcium phosphate apatite	-	-	9	6.5
Calcium carbonate	6	21.4	34	24.5
MISC Materials	-	-	4	2.9
Total	**28**	**100**	**139**	**100**

Figure 1—Photograph of magnesium calcium phosphate carbonate uroliths from an intact male, 2 years 3 months Hampshire sheep breed. Measurements are shown in centimeters.

CHAPTER 10

Epidemiological evaluation of calcium carbonate uroliths in horses.

SUMMARY

This cross-sectional retrospective case study evaluated urolith submissions data from 346 horses with calcium carbonate ($CaCO_3$) uroliths. Uroliths were submitted to the Minnesota Urolith Center (MUC) between January 1, 1981 and December 31, 2014. The objective of this study was to determine whether the frequency of occurrence of $CaCO_3$ uroliths in horses varied with breed, age, sex, reproductive status, anatomic location within the urinary tract, season of uroliths collection, and geographic location of urolith-formers; and to determine whether the rates of $CaCO_3$ urolith submitted to the MUC varied over time. We hypothesize that horses of a particular breed, age, sex and reproductive status, in certain season and geographic location in the USA are associated with the frequency of $CaCO_3$ urolith formation. The Mean ± SD age of 346 horses with $CaCO_3$ was 13 ± 1.8 years, and ranged from 0.3 to 36 years. Quarter horse, Thorough Bred and Arabian breeds comprised 173 of 292 (59%) of the horses with $CaCO_3$ urolith. Horses between the ages of 6 and 17 comprised 179 of 313 (57%) animals that had $CaCO_3$ uroliths. Male horses [237 of 331 (72%)] had higher proportion of $CaCO_3$ uroliths compared to females [94 of 331(28%)]. Neutered horses [199 of 331(60%)] had more $CaCO_3$ uroliths compared with sexually intact [132 of 331(40%)]. More $CaCO_3$ uroliths were retrieved from the lower portions of the urinary tract [276 of 323 (85%)] compared to the upper portions of the urinary tract [44 of 323 (14%)], and upper/lower portions [3 of 323 (< 1%)] of horses. Between 1981 and 1997, [95% (76 of 80)] of $CaCO_3$ were confirmed in horses compared to [5% (4 of 80)] of other minerals. Between 1998 and 2014 $CaCO_3$ comprised [97% (270 of 278)] while other minerals comprised [3% (8 of

278)]. Awareness of the prevalence of $CaCO_3$ uroliths along with knowledge of the etiologic, demographic, and environmental risk and protective factors for urolithiasis may facilitate development of surveillance strategies that result in earlier detection of urolithiasis in horses. Thus, prevention of urolithiasis in horses should target these potential risk and protective factors. Modification of risk factors may help to minimize urolith formation, dissolve existing uroliths, and/or minimize urolith recurrence.

INTRODUCTION

Uroliths are macroscopic concretions that form within the urinary tract. They are primarily composed of minerals filtered by the kidneys, but can be formed from any substances that are sparingly soluble in urine. The prevalence of equine urolithiasis has been estimated to be low: 0.04 to 0.5% from studies performed in different equine clinics[136] and 0.7% in slaughtered horses in Spain.[137] Urolithiasis has been recognized in horses for more than a century but the pathophysiology of equine uroliths formation is not fully understood.[138]

The purpose of this study is to determine the prevalence of naturally occurring $CaCO_3$ uroliths retrieved from horses and submitted to the MUC between January 1, 1981 and December 31, 2014 and to determine whether breed, age, sex, reproductive status, anatomic location within the urinary tract, geographic location within USA and season of uroliths detection were risk factors associated with $CaCO_3$ urolith formation.

MATERIALS AND METHODS

Identification of cases and medical records review—Medical records of the Minnesota Urolith Center[a] were reviewed to identify horse uroliths submitted between January 1, 1981 and December 31, 2014. The mineral composition of the uroliths was determined by optical crystallography [42-43] and, when necessary, by infrared spectroscopy.[79] Cases were identified as horses that had uroliths consisting ≥ 70% $CaCO_3$. Compound horse uroliths (i.e., those containing nuclei, stone, shell, or surface crystal layers with ≥ 2 layers with < 70% different mineral types were excluded.

The following information were retrieved from urolith submission records of each horse when applicable: breed, age, sex, reproductive status (neutered or sexually intact), state of residence of urolith-formers, and date of urolith submission. Regions in the USA (southeast, southwest, mid-west, northeast, or west) were determined on the basis of state of residence according to classification established by the National Geographic Education division.[c] Seasons of sample collection were classified as winter (December, January, February), spring (March, April, May), summer (June, July, August), and fall (September, October, November). The location of uroliths retrieved from the kidneys or ureters were classified as upper tract uroliths, and uroliths retrieved from the bladder and the urethra were classified as lower tract uroliths. If uroliths were submitted more than once for the same animals, only the first submission was included in the analysis.

Methods of quantitative urolith analysis – Uroliths from horses in this study were analyzed by optical crystallography[42-43] and Fournier transform infra-red spectroscopy.[79]

Statistical analysis—Standard statistical software[44 e f g k,] was used to determine descriptive statistics (mean, median, and SDs) of breed, age, breed, sex, reproductive status, geographic location of horses in the USA, season of urolith submission, and anatomic location of the uroliths within the urinary tract for case animals. Because we were unable to obtain reliable controls without uroliths, we are only able to report descriptive statistics. For analysis of age effects, animals of 1 year, 1 to < 6 years, 6 to < 12 years, 12 to < 18 years, 18 to < 24 years, 24 to < 30 years and 30 to > 36 years, horses were divided into 6 age groups. To obtain more specific data on age effects, data for horses < 18 years of age were also compared to horses' ≥ 18 years old.

Prevalence of $CaCO_3$ uroliths was expressed as the relative frequency of all horse uroliths submitted for analysis to the MUC. Relative frequencies also were used to describe the age, breed, sex, reproductive status, and geographic[j] location of horses, as well as season[h] of uroliths collection and anatomic location of uroliths. The 28-year study was grouped into 2 intervals [(1981 through 1997) and (1998 through 2014)] to determine whether the rates of urolith submissions changed between these two periods.

Risk factor is a variable associated with an increased occurrence of disease or infection. In this study risk is defined as the increased occurrence of $CaCO_3$ uroliths in horse. Therefore, risk can be computed as follows:

$$\textbf{Risk} = \frac{\textbf{Number of horses with } CaCO_3 \textbf{ uroliths}}{\textbf{Number of horses exposed to } CaCO_3 \textbf{ uroliths.}}$$

This study did not use a comparison group without uroliths. Therefore, this study will use proportion or frequency as the primary measure of urolith risk in horses. Although, we could not obtain reliable controls, they are expected to be exposed to the same risk factors.

RESULTS

Between January 1, 1981 and December 31, 2014 uroliths from 358 horses' resident in the USA were submitted to the MUC for analysis during the study period. Of these, uroliths containing ≥ 70 % of $CaCO_3$ received from United States regions were 346 of 358 $CaCO_3$ (97%; **Table 1**). Calcium carbonate contents of the 346 uroliths were as follows: 100%, 335 of 346 (97%) samples; and ≥ 70% to 99%, 11 of 346 (3%). Calcium carbonate uroliths retrieved from horses were typically roughly circular and mosaic/ speculated surface/rough surfaced in shape. They were brown; clotted-blood intermingled with yellow color and range from 1 mm to 2 cm in diameter (**Figure 1**). Equine $CaCO_3$ urolith submissions to the MUC progressively increased from 80 in the period of 1981 to 1997; to 278 in the period of 1998 to 2014.

Breed—Breed data were provided for 292 of 346 (84%) horses with $CaCO_3$ uroliths submitted to the MUC. Horses identified with $CaCO_3$ uroliths in our study included 31 breeds. Breed data for 54 of 346 (16%) case horses were not provided. Of the 292 urolith submissions where breeds were recorded, 106 of 292 (36%) were Quarter horses, 45 of 292 (15%) were Thorough bred and 22 of 292 (8%) were Arabians. Twenty-eight of the other horse breeds comprised 119 of 292 (41%) of animals with $CaCO_3$ (**Table 2**).

Age— Age data for 313 of 346 (90.4%) horses were recorded. The Mean ± SD age of horses was 13 ± 1.8 years and ranged from 0.3 to 36 years. Two hundred-thirty-five of 313 (75%) horses were < 18 years and 78 of 313 (25%) were ≥ 18 years of age. Horses < 1 year of age comprised 7 of 313 (2%); 1 to < 6 years comprised 49 of 313 (16%); 6 to < 12 years comprised 87 of 313 (28%), 12 to < 18 years comprised 92 of 313 (29%), 18 to < 24 years comprised 54 of 313 (17%), 24 to < 30 years comprised 23 of 313 (7%) and 30 to > 36 years comprised 1 of 313 [(< 1%); **Table 3**].

Sex and reproductive status—Of the 346 horses, sex was recorded for 331 of 346 (96%). Two hundred thirty-seven of 331 (72%) horses were males and female were 94 of 331 (28%). Reproductive status for 331 of 346 (96%) horses was recorded. One hundred and ninety-nine of 331 (60%) were neutered and 132 of 331 (40%) were sexually intact. A higher proportion of male horses had $CaCO_3$ uroliths than females. A higher proportion of neutered horses had $CaCO_3$ uroliths than sexually intact (**Table 3**).

Seasonal distribution—Submission of 346 horses uroliths took place as follows: 101 of 346 (29%) in fall, 78 of 346 (22%) in summer, 82 of 346 (24%) in winter, and 85 of 346 (25%) in spring. Calcium carbonate uroliths in horse were mostly submitted in fall than in other seasons (**Table 3**).

Geographic distribution— Of the 346 horse uroliths, 24 of 346 (7%) were received from the northeast, 65 of 346 (19%) from the southeast, 19 of 346 (5%) from the southwest, 154 of 346 (44.5%) from the mid-west, and 82 of 346 (24%) from the west. In 2 of 346 (< 1%) the geographic locations were not recorded. There were more horse $CaCO_3$ uroliths received from the mid-west region than from every other region (**Table 3).**

Anatomic location—Anatomic locations were reported for 323 of 346 (93%) horse uroliths and unknown for 23 of 346 (7%). Two hundred and seventy-six of 323 (85%) horses had urolith samples obtained from the lower portions of the urinary tract (urinary bladder, urethra, voided uroliths, or some combination of these). Uroliths from 44 of 323 (14%) were obtained from the upper portions of the urinary tract (kidney or ureter). Uroliths obtained from both upper and lower urinary tracts comprised 3 of 323 (< 1%). Uroliths obtained from the bladder only were [145 of 323 (45%)] and from the urethra only were [98 of 323 (30%; **Table 5**)]. A greater proportion of the uroliths were obtained from the bladder than from the urethra. Two hundred thirty-seven of 346 (69%) uroliths were located in the urinary tract of males, 94 of 346 (27%) in the urinary tract of females and in 15 of 346 (4%) the anatomic locations were not reported. More $CaCO_3$ uroliths were retrieved from the urinary tract of males than female horses.

Diet—The primary types of diet used was recorded in [125 of 346 (36%)] horses. In 84 of [125 (67%)] horses the diets were a combination of grass hay/ alfalfa hay/Timothy hay. In [221 of 346 (64%)] the type of diet used was not recorded.

Method of retrieval— Of the 346 horse with $CaCO_3$ uroliths submitted to the MUC, the retrieval method for 284 (82%) were not reported. Of the 62 episodes for which the method of retrieval was reported, [2 of 62 (3.2%)] was by basket retrieval, [1 of 62 (1.6%)] by catheter retrieval, [4 of 62 (6.5%)] by lithotripsy, [19 of 62 (30.6%)] by necropsy, [1 of 62 (1.6%)] were obtained by owners, [27 of 62 (43.5%)] were by surgery, and [8 of 62 (12. 9)] were by voiding. The remaining 284 of 346 (82.1%) retrievals were not reported.

Urolith submission rate changes over time—The rates of $CaCO_3$ uroliths retrieved from horses and submitted to the MUC increased from 95% (1981-1997) to 97% (1998-2014) compared to other minerals retrieved from horses during the same period with 5% and 3% respectively (**Table 7**).

DISCUSSION

The results supported our hypothesis that multiple risk factors such as breed [31% (106 of 346)], age [27% (92 of 346)], sex [69% (237 of 346)], reproductive status [585 (199 of 346)], geographic location [45% (154 of 346)], and season of urolith submission [29% (101 of 346)] likely played a role in the formation of $CaCO_3$ uroliths. Our reports were in agreement with reports by other investigators.[136, 140,141]

As theorized in urolith formation in other species, it is possible that when several risk factors occur together, their individual effects may be enhanced. These risk factors were grouped as follows: etiological (infectious and toxins), demographic (breed, age, sex, and genetic) and environmental (environmental, water and food sources and socioeconomic status). Thus, each risk factor or protective factor may play either a limited or a major role in the formation or prevention of $CaCO_3$ uroliths [77]The animals in the present study most likely had clinical signs. Our study may not have included asymptomatic animals, horses that voided uroliths uneventfully, or those whose uroliths were not collected and submitted to the MUC for analysis. Horses that died as a result of urinary tract obstructions and were euthanized but not necropsied or horses from geographic locations where other urolith analysis laboratories may have solicited submissions were not represented. As a result of these limitations, this study may not have fully represented the population of all horses with $CaCO_3$ uroliths.

The number of horses with $CaCO_3$ uroliths in this study reflected an increased awareness in owners that uroliths can cause urethral obstruction and/or increased use of diagnostic methods to detect uroliths such as survey radiography. Submission rates may also have been associated with an increased awareness of the availability of complimentary urolith analysis at the MUC. The results of our study indicated that the proportion of Quarter horses with $CaCO_3$ uroliths [106 of 292 (36%)] was higher than other breeds. Familial predisposition for urolithiasis has been well documented in various species for some mineral types.[80] For example, genetic mutations causing cystine urolithiasis have been identified in dogs [70] and humans.[71]To the authors' knowledge a genetic predisposition for $CaCO_3$ urolithiasis in horses has not been explored. Prospective studies evaluating the effects of diet, housing, body condition score, and other potential risk and protective factors in addition to breed are necessary to further investigate a potential predisposition to this type of urolithiasis.

In our study, age was associated with $CaCO_3$ uroliths formation in horses, with Mean $\pm$ SD age being 13 $\pm$ 1.8 years and horses between the ages of 6 and 18 years having more $CaCO_3$ uroliths (63%) compared to other age groups (**Table 3**). Similarly, horses that were $<$ 18 years had a higher proportion of $CaCO_3$ urolith (75%) compared to horses $\geq$ 18 years (25%). Epidemiological data derived from studies [55-57] of dogs, cats, and humans suggested that advancing age is a risk factor for urolithiasis until decline sets in at the age of ≥ 18. This is not surprising considering that the normal average life span of a horse is between 25 to 33 years and mean age of 13 ± 1.8 years.

Our results also indicated that the proportion of $CaCO_3$ uroliths were greater in males (237 of 331; [72%]) compared to females (94 of 331; [28%]). In (15 of 346 [4%]) horse; the sexes were not recorded. Our study had depicted that $CaCO_3$ uroliths in horses occur primarily in males compared to females. This is likely due to the anatomical and functional differences in the urinary tract of male and female horses. This is likely related to the fact that males' urethras are substantially longer and narrower than those of females.[82] In stallions or gelding, blockage is more common because the urethra is longer and there is a narrowing of the urethra where the penis passes out of the pelvic canal and curves downward. It is likely that $CaCO_3$ uroliths are not commonly detected in mares because the larger/distensible and shorter urethra allows uroliths to be passed in the urine stream before they can cause clinical signs. A higher proportion of horse [199 of 331 (60%)] were neutered compared to sexually intact horses with [132 of 331 (40%); **Table 3**].

The proportion of $CaCO_3$ uroliths we have observed in neutered horses was greater compared to sexually intact. This observation is similar to what we have observed in goats and ferrets.[78, 117]This observation supports other studies that have suggested that neutering may be a risk factor for urolithiasis. One group of investigators has generated convincing evidence that early neutering may result in underdevelopment of the urethra and thus predispose adult horses to urethral obstruction. [83]The same group of investigators have shown that neutering reduces production of testosterone, which normally aids in the development of urethral lumen. A reduced circulating testosterone concentration is also believed to diminish normal preputial-penile attachments.[83]These factors have been incriminated to increase the frequency of urolith formation in horses. Because the ages at which horses in this study were neutered was not provided, we were unable to determine whether early neutering is associated with the frequency of $CaCO_3$ urolith formation.

Two hundred and seventy-six of 323 (85%) horse uroliths in this study were retrieved from the lower portions of the urinary tract (**Tables 4**). The predominant location of uroliths in the lower portions of the urinary tract of other domestic animals has been described.[24] In our experience in other species, the development of uroliths in the upper urinary tract is likely underreported because survey radiography and ultrasonography are often times omitted during initial evaluation of animals with clinical signs of urolithiasis. Clinical signs caused by uroliths in the lower portions of the urinary tract (increased voiding frequency, straining to void, and dribbling urine) are more likely to be observed than are clinical signs caused by uroliths located in the upper urinary tract (polyuria and hematuria without obvious signs of pain, which may go unrecognized). In animals of the present report, uroliths were less commonly retrieved from the urethra only which comprised 98 of 323 (30%) compared to bladder only that was 145 of 323 (45%). These localizations are important when considering dietary modification for dissolution of uroliths. It has been recommended that attempts to dissolve urethral and ureteral uroliths by dietary modification be avoided.[24] Uroliths lodged in the ureters or urethra cannot be dissolved by medical protocols because urolith dissolution requires sustained contact with urine that has been modified and therefore is undersaturated with lithogenic mineral.[84] The good news is that the uroliths dissolution in horses is very feasible because there are more bladder uroliths compared to urethral and ureteral uroliths.

In our study, the proportion of $CaCO_3$ uroliths detection in horses residing in the mid-western region of the US was higher compared to horses residing in the other regions. These observations indicate that geographi**c** location may increase the frequency of urolithiasis. Geographic prevalence of urolithiasis has been reported in dogs and humans. [87-88]Our study was not designed to determine the reason for the variations among geographic locations. Further research is needed to assess the factors that contribute to such differences.

Calcium carbonate uroliths were mostly retrieved from horses in fall than in other season. The association between season and detection of urolithiasis is in agreement with findings of other investigators, who reported that detection of urolithiasis in cats, sheep, and humans varies with season.[87-89] It is probable that reduced water intake during the fall season could have led to increased urine concentration. Concentrated urine is a risk factor for urolith formation. We did not investigate possible associations between $CaCO_3$ urolithiasis and these factors.

Diet has been incriminated as important risks factor for $CaCO_3$ uroliths formation in horses.[90] In our study; we encountered a lack of detailed information regarding diet composition and variability in the reported diets fed to the horses. Lack of consistency in response to our requests for dietary history prevented evaluation of dietary components as risk factors for this disease. To our knowledge, controlled, blinded clinical studies have not been performed to evaluate associations between most dietary risk factors and urolith formation in horses. Our study was also not designed to explore this factor, and we were unable to test for such associations in horses. In our experience in other species it is probable that diet has a role to play in the pathogenesis of $CaCO_3$ urolithiasis. Most case horses for which diet was reported primarily were fed grass; Timothy hay and alfalfa hay 84 of 125 (67%). Further prospective studies are needed to define the role played by each of these risk factors singly and in combination as they relate to $CaCO_3$ urolithiasis.

Healthy horses normally excrete urine that has characteristic gray white color, pungent odor, and is alkaline, with a pH range of 7.5 to 8.5. [91]Equine urine has the disadvantage of being highly laden with calcium crystals and in the presence of highly alkaline urine may favor crystallization of crystals and subsequent $CaCO_3$ urolith formation.[91] Sheep, goats, rabbits, and guinea pigs also normally excrete alkaline urine. Production of alkaline urine is shared characteristics among these herbivorous species. Most of the plants used to feed horses in this study (e.g., grass and alfalfa) are associated with alkaline urine production.[91] Although, not investigated in the present study, it is probable that alkaline urine pH is a risk factor for $CaCO_3$ urolithiasis in horses, and this should be investigated in future studies.

Calcium carbonate uroliths comprised 346 of 358 (97%) of all uroliths retrieved from horses' resident in the USA in this study. Every other urolith retrieved from horses in this study combined was 3%. This observation is not surprising because normal horse's urine is usually gray-white and/ or thick, containing mucus (which make it cloudy) and calcium crystals. It has been proved that the cloudiness that is usually observed in normal horse urine is as a result of calcium crystals mainly calcium carbonate that are being excreted by the kidneys. Unlike in humans, horses tend to absorb excessive amount of calcium from the intestine so that excesses have to be eliminated through the urine.[141]This is not surprising because the main component of equine urolith is $CaCO_3$. Urolith formation in horses usually results from damage to the renal parenchyma as well as uroepithelium of the ureters, bladder or urethra. Mucus excretions from the kidneys of horses protect the ureteral, bladder and urethral mucosa from calcium crystal deposition and possible damage.[142-144]But when the linings of these organs are compromised in addition to presence of suitable alkaline urine pH, calcium carbonate uroliths formation will ensure. Further studies are needed to substantiate this observation.

In our, the primary methods of $CaCO_3$ uroliths retrieval in horses were by surgery (n=27), necropsy (n=19), void (n-=8), lithotripsy (n=4), basket retrieval (n=2), and catheter retrieval (n=1) and owner obtained (n=1). All in all, basket retrieval, lithotripsy, catheter retrieval, and surgery comprised 34 of 62 (55%) uroliths retrieval methods reported. These manipulations often times lead to traumatization of uroepithelium and necrotic debris production which may provide a nidus for $CaCO_3$ urolith formation. This is not surprising because equine urine has the disadvantage of being highly laden with calcium crystals as well as highly alkaline thereby favoring crystallization and subsequent $CaCO_3$ urolith formation. Similarly, in this study necropsy comprised 19 of 62 (31%) of horse whose retrieval methods that were reported thereby emphasizing the risk of urinary tract obstruction in horses due to $CaCO_3$ uroliths that were retrieved through necropsy. Veterinarians should be on the lookout for $CaCO_3$ urolihiasis in horses because of the possibility of urinary tract obstruction.

Conclusion

The Mean ± SD age of 346 horses with $CaCO_3$ was 13 ± 1.8 years, and ranged from 0.3 to 36 years. Quarter horse, Thorough Bred and Arabian breeds comprised 173 of 292 (59%) of the horses with $CaCO_3$ urolith. Horses between the ages of 6 and 17 comprised 179 of 313 (57%) horses with $CaCO_3$ urolith. Male horses had higher proportion of animals with $CaCO_3$ uroliths compared to females. Neutered horses had higher frequency of $CaCO_3$ urolith than sexually intact. More $CaCO_3$ uroliths were retrieved from the lower portions of the urinary tract of horses (85%) compared to the upper portions of the urinary tract (14%), and upper/lower portions (< 1%) of horses. Between 1981 and 1997, 95% of $CaCO_3$ were confirmed in horses compared to 5% of other minerals. Between 1998 and 2014 $CaCO_3$ comprised 97% of horse uroliths while other minerals comprised 3%.

Although information derived from the extensive urolith database compiled by the MUC[a] provides a unique resource for retrospective evaluation of the epidemiological features of this disorder, it is important to note that cross-sectional retrospective case studies do not prove cause-and-effect relationship. Additional prospective or interventional studies are needed to prove hypotheses derived from such studies.

Knowledge of the predominant mineral types of uroliths in horses, 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 horses**.** Determination of the frequency of $CaCO_3$ urolith in horses may help to minimize oversaturation, prevent urolith formation, and/or minimize recurrences. Similarly, knowledge of the epidemiology of urolithiasis is essential for constructing effective experimental designs and choosing appropriate cases and controls for conducting clinical trials with meaningful results that may facilitate development of surveillance strategies that result in earlier detection of uroliths in horses.

Biases and limitations. Since our data was obtained from horse urolith submissions to the MUC, it was not a complete representative of all urolith submissions from every breed, age, sex, season and geographic location of all horse populations.

In our study $CaCO_3$ uroliths in horses occur primarily in males compared to females. In stallions or gelding, urinary tract blockage is more common because the urethra is longer and there is a narrowing of the urethra where the penis passes out of the pelvic canal and curves downward. All these features may have contributed to more clinical manifestations of urethral obstruction in males compared to females and may have therefore influenced their selection and subsequent submission to the MUC as cases. Uroliths are not commonly detected in mares because the larger/distensible and shorter urethra allows uroliths to be passed in the urine stream before they can cause clinical signs.

Since the MUC uroliths analyses are performed at no cost to veterinarians and/ or owners this may have influenced the rate of urolith submissions to the center. Also because the sample size of cases in this study was not very large we were unable to apply the conclusions derived from the findings of our study to the whole horse populations.

Uroliths obtained from male horses in this study were retrieved mainly from the lower portions of the urinary tract compared to the upper portions of the urinary tract. Clinical signs are more likely to be observed when uroliths are located in the lower portions of the urinary tract than from the upper portions of the urinary tract. This may have constituted a form of bias in this study leading to higher number of lower urinary tract uroliths being observed in this study compared to uroliths retrieved from the upper portions of the urinary tract.

This study also may have included only clinical cases, and may not have included asymptomatic cases or cases that may have been euthanized due to complications associated with urinary tract obstructions without a subsequent necropsy; as a result, this may have limited the use of the result of the study reported here to fully represent the population of every horse with $CaCO_3$ uroliths and therefore may have constituted a form of bias.

Table 1--Mineral composition of 167 uroliths retrieved from sheep resident in the USA and submitted to the Minnesota Urolith Center between 1981and 2014.

Variable	**Number of uroliths**	**%**
Calcium carbonate	346	96.6
Calcium oxalate (COM)	2	< 1
Compound	6	1.7
MAP (struvite)	1	< 1
Magnesium calcium phosphate carbonate	1	< 1
MISC Materials	1	< 1
Mixed	1	< 1
Total	**358**	**100**

Table 2—Breed distribution for 346 horses with $CaCO_3$ uroliths resident in the USA and submitted to the MUC between 1981 and 2014.

Breeds	**Number**	**Per cent**
Appaloosa	14	4
Arabian	22	6.4
Belgian	1	< 1
Donkey	6	1.7
Egyptian Arab	1	< 1
Hanoverian	4	1.2
Holsteiner	1	< 1
Mangalarga Marchado	1	< 1
Miniature Donkey	6	1.7
Miniature Horse	7	2
Missouri Fox Trotter	2	< 1
Mixed	7	2
Morgan	4	1.2
Mule	2	< 1
Mustang	2	< 1
Oldenburg	1	< 1
Paint	16	4.6
Paso Fino	5	1.4
Peruvian Paso	1	< 1
Pony	6	1.7
Przewalski	1	< 1
Quarter Horse	106	30.6
Racking	1	< 1
Rocky Mountain Horse	1	< 1
Saddle Bred	1	< 1
Spotted Saddle Horse	1	< 1
Standard Bred	8	2.3
Tennessee Walker	7	2
Thorough Bred	45	13
Trakehner	4	1.2
Unknown	54	15.6
Westphalian	1	< 1
Total	**346**	**100**

Table 3—Summary data for 346 uroliths retrieved from horses' resident in the USA and submitted to the MUC between 1981 and 2014 indicating proportions for age, combined age category, sex and reproductive status, season and geographical location.

Variables	Description	Number of cases	Per cent
Age	< 1 year	7	2
	1 – < 6 years	49	14.2
	6 - < 12 years	87	24.1
	12 - < 18 years	92	26.6
	18 - < 24 years	54	15.6
	24 - < 30 years	23	6.6
	30 > 36 years	1	< 1
	Unknown	33	9.5
Total		**346**	**100**
Combined age category			
	≥ 18 years	78	22.5
	< 18 years	235	67.9
	Unknown	33	9.5
Total		**346**	**100**
Sex			
	Males	237	68.5
	Females	94	27.2
	Unknown	15	4.3
Total		**346**	**100**
Reproductive status			
	Neutered	199	57.5
	Sexually intact	132	38.2
	Unknown	15	4.3
Total		**346**	**100**
Season			
	Fall	101	29.2
	Spring	85	24.6
	Summer	78	22.5
	Winter	82	23.7
Total		**346**	**100**
Geographic location	Midwest	154	44.5
	Northeast	24	6.9
	Southeast	65	18.8
	Southwest	19	5.5
	West	82	23.7
	Unknown	2	< 1
Total		**346**	**100**

Table 4— Anatomic locations for 346 CaCO3 uroliths retrieved from horse and submitted to the MUC between 1981 and 2014.

Anatomic location	Number	%
Bladder	145	41.9
Bladder/urethra	13	3.8
Bladder/Urethra/Void	1	< 1
Kidney	34	9.8
Kidney/Bladder	1	< 1
Kidney /Ureter	3	< 1
Kidney/Ureter/bladder	1	< 1
Ureter	7	2
Ureter/Bladder	1	< 1
Urethra/Void	2	< 1
Urethra	98	28.3
Void	17	4.9
Unknown	23	6.6
TOTAL	**346**	**100**
Lower tract	276	79.8
Upper tract	44	12.7
Lower/Upper tract	3	< 1
Unknown	23	6.6
Total	**346**	**100**

Table 5—Different types of diets used to feed 346 horses with $CaCO_3$ uroliths resident in the USA and submitted to the MUC between 1981 and 2014.

Diet	Number	Per cent
Grass/Alfalfa /Timothy hay	84	24.3
Equine SR	5	1.4
Grain	2	< 1
Pasture	11	3.2
Pellet	2	< 1
Sweet feed	7	2
Other types of feed not specified	14	4
Unknown	221	63.9
Total	**346**	**100**

Table 6—Method of retrieval for 346 $CaCO_3$ uroliths retrieved from horse's resident in the USA and submitted to the MUC between 1981 and 2014.

Method of retrieval	Number	Per cent
Basket	2	< 1
Catheter	1	< 1
Lithotripsy	4	1.2
Necropsy	19	5.5
Owner obtained	1	< 1
Surgical	27	7.8
Voided	8	2.3
Unknown	284	82.1
Total	**346**	**100**

Table 7—CaCO3 uroliths compared to other minerals retrieved from horses resident in the USA and submitted to the MUC between 1981 and 2014.

Stone Type	**Year of submission**			
	1981-1997		1998-2014	
	No.	%	No.	%
Calcium carbonate	76	95	270	97
Calcium oxalate	2	2.5	-	-
Struvite (MAP)	1	1.2	-	-
MISC Materials	1	1.2	-	-
Compound	-	-	6	2.3
MCPC	-	-	1	< 1
Mixed	-	-	1	< 1
Total	**80**	**100**	**278**	**100**

Figure 1—Photograph of calcium carbonate uroliths (100% CaCO3) from a 10-year-old neutered male horse. Measurements are `shown in centimeters.

FOOT NOTES

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

b. Veterinary Medical Database, 1717 Philo Road, Suite 15, Urbana, IL 61803-3007.

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

d. One-piece tissue Cassette. 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 Street. SE, Minneapolis, MN 55414.

f. EPI INFO, version 6.04b, Center for Disease Control, Atlanta, GA. Available at www.cdc.gov/Epiinfo/. Accessed, December 30, 2016.

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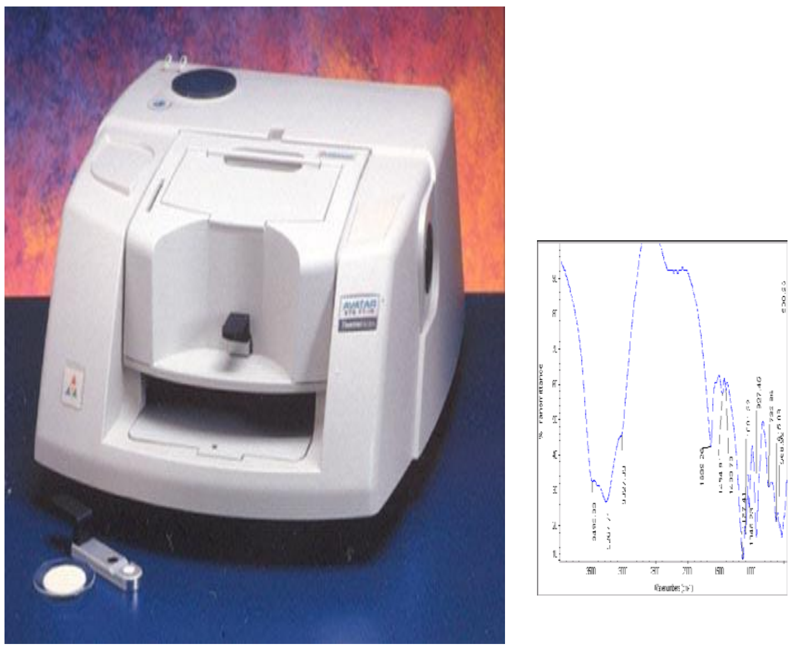
APPENDICES

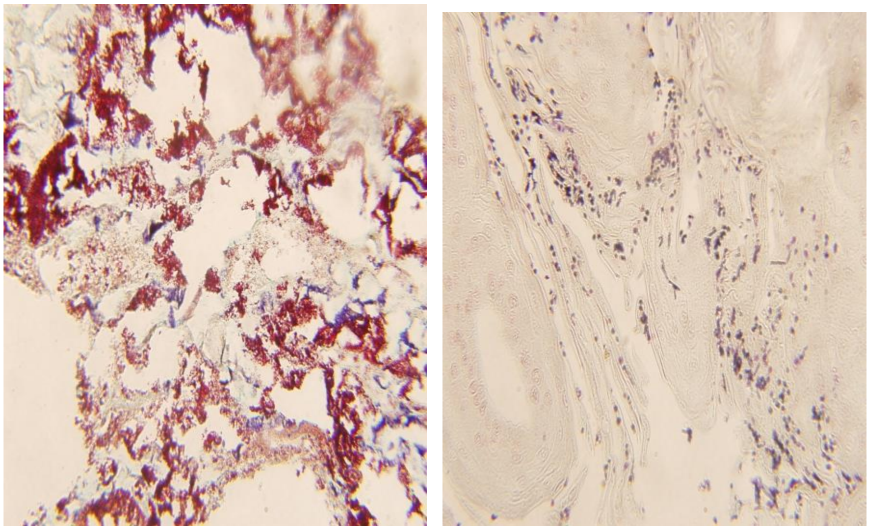
Appendix A – Figure 1- Optical crystallography for urolith analysis

Appendix A – Figure 2-Infrared spectroscopy for urolith analysis

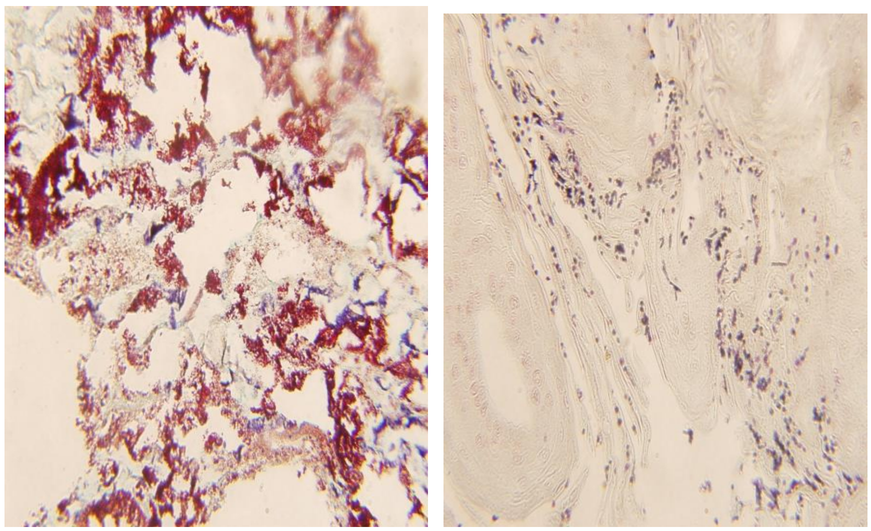


Appendix A- Figure 3- Ferret struvite urolith after washing (Arrowed) and grinding

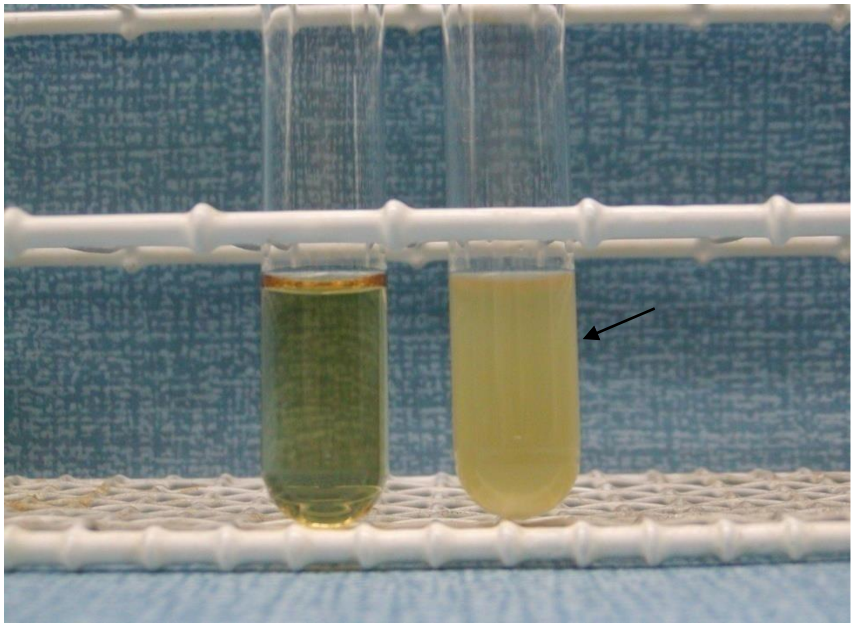
Appendix A- Figure 4- Negatively H& E stained struvite urolith (left) and a positive control (right; Arrowed).



Appendix A- Figure 5- Negative Gram stained struvite urolith (left) and the positive control (right; Arrowed).



Appendix A – Figure 6- Negative ferret struvite urolith culture (Left) and Positive control (Right; Arrowed)



Appendix A- Figure 7- Negative struvite urolith on blood agar (Left) and positive control on the right)

NEGATIVE POSITIVE

Appendix A- Figure 8-Negative struvite uroliths culture on MacConkey agar (Left) and positive control (right; Arrowed).

NEGATIVE POSITIVE