

**Szent István University**  
**Postgraduate School of Veterinary**  
**Science**

**Epidemiological evaluation of canine and  
feline urolithiasis in Hungary**

PhD thesis

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## Table of contents

1	Introduction .....	5
2	Materials and Methods.....	7
2.1	Sample and population data.....	7
2.1.1	Canine samples .....	7
2.1.2	Feline samples.....	7
2.1.3	Population data.....	7
2.2	Urolith analysis .....	8
2.3	Genetic test .....	8
2.4	Statistical analysis .....	9
3	Results .....	10
3.1	Characteristics of the epidemiology of canine urolithiasis and the affected breeds in the dog population of Hungary .....	10
3.2	Examination of the prevalence of the genetically determined hyperuricosuria in the dogs with purine urolithiasis .....	12
3.3	Epidemiology of urolithiasis in cats .....	13
4	Discussion.....	14
4.1	Eidemiology of canine urolithiasis .....	14
4.1.1	Epidemiologic data .....	14
4.1.2	The affected breeds.....	15

4.1.3	Struvite urolithiasis.....	15
4.1.4	Oxalate urolithiasis .....	16
4.1.5	Purine urolithiasis .....	17
4.1.6	Cysine urolithiasis.....	18
4.1.7	The incidence of urolithiasis .....	19
4.1.8	Change of the prevalence of the mostly affected breeds and its' effect on the occurrence of urolithiasis.....	20
4.2	Examination of the genetic background of the purine urolithiasis in dogs.....	22
4.3	Epidemiology of urolithiasis in cats .....	24
5	New scientific results .....	26
6	Scientific publications of the thesis .....	28
7	Acknowledgements.....	30

## **1 Introduction**

Diseases and special metabolic conditions can cause crystallisation of stones of different chemical composition in the urinary tract. There is a strong linkage between the composition of the stone and the nature of the underlying factor or disease. The prevalence of different type of stones vary in respect of species, breed, gender or age. Furthermore there are significant differences when one compare the results of geographically different studies. The statistical evaluation of the current epidemiologic data on urolithiasis can reveal the presence of inherited diseases and predispositions. This knowledge can help to determine the group of animals or breeds at risk to a special kind of stone disease. Also the consideration of breeding strategies and screening tests in an affected breed can help to prevent the spreading of stone diseases in the population.

The aim of this study was to describe the current epidemiology of canine and feline urolithiasis in Hungary and to asses the incidence rate of urolithiasis in dogs. Based on the epidemiologic data the authors intended to

define breeds at risk for different types of urolithiasis and to identify the genetic background too in some affected breed.

## **2 Materials and Methods**

### **2.1 Sample and population data**

Analysis of canine and feline uroliths from Hungary sent to the laboratory of the Budapest Urolith Centre (BUC) with a standard data questionnaire containing species, breed, age, gender as well as clinical data from 2001 to 2014 were conducted.

#### **2.1.1 Canine samples**

A total of 2,543 canine samples were submitted and analysed between the years 2001 and 2012.

#### **2.1.2 Feline samples**

A total of 480 feline samples were submitted and analysed between the years 2006 and 2014.

#### **2.1.3 Population data**

Population data were gathered from a previous report on the dog population of Budapest (n= 124,459) and from the Hungarian Microchip Register (n=543,929). The number of dogs living in the countryside was estimated upon the data provided by the Animal Welfare Directorate of the National Food Chain Safety Office

(NFCSSO) in the years 2010, 2011 and 2012, respectively.

Data on the cat population of Hungary was not available.

## **2.2 Urolith analysis**

After macro- and microscopic evaluation of the samples a quantitative or semi-quantitative evaluation of the chemical composition of each different layers of uroliths were processed by ultra-microchemical (UMC) method (Reanal, Hungary) and in part by infrared spectroscopy (Perkin Elmer 1600 FTIR). Uroliths were classified upon the predominant mineral component that exceeded the 70% of the stone mass.

## **2.3 Genetic test**

DNA sample were gathered from whole blood (Qiagen Genra Puregene Blood Kit, Qiagen, Germany). The DNA preps were genotyped for the presence of a missense mutation (Cys181Phe) in the gene SLC2A9 in linkage to the hyperuricosuria (HUU). The sequencing



was processed in the University of California, Veterinary Genetics Laboratory, USA.

## **2.4 Statistical analysis**

The proportion of affected breeds for each urolith type was compared to the proportion of crossbreed dogs. Odds ratio (OR) and significance level were calculated by logistic regression by the means of using the R statistical software (R Core Team, 2013). Statistical significance level was considered at 5% ( $p < 0.05$ )

### **3 Results**

#### **3.1 Characteristics of the epidemiology of canine urolithiasis and the affected breeds in the dog population of Hungary**

In the study period (from 2001 to 2012) the 64 % of the the total of 2,543 samples were composed of struvite, 15,5 % calcium-oxalate, 13,1 % purine and 4,2 % cystine.

The maen age of dogs affected by struvite urolithiasis was 76,2 months, an those of oxalate urolithiasis was 106,8 months.

The ratio of affected males to females was 0,6, in struvite, 7,1, in oxalate and 13,3 in purine urolithiasis respectively. There was no affected female observed within the cystine stone forming dogs.

The average incidence rate of a 3-year period (2010-2012) was 1.76/10,000.

99 breeds were involved in the study.

Based on the statistical evaluation of the epidemiologic data the breeds affected more than one type of urolithiasis are the Miniature Pinscher oxalate (OR 5,4  $p < 0,001$ ) and cystine (OR 12,7,  $p < 0,001$ ),

Yorkshire Terrier oxalate (OR 3,5  $p < 0,001$ ) and purine (OR 3,1  $p < 0,001$ ), English Bulldog purine (OR 17,1  $p < 0,001$ ) and cystine (OR 18,6  $p < 0,001$ ) urolithiasis. Breeds affected by *struvite stone diseases* are the Bernese Mountain dog (OR 11,1  $p = 0,018$ ), the German Shephard (OR 8,8  $p = 0,002$ ), the Shi Tzu (OR 2,5  $p = 0,007$ ) and the English Cocker Spaniel (OR 2,0  $p = 0,019$ ); *oxalate* occurs more frequently in Miniature Poodle (OR 3,2  $p = 0,008$ ) and Miniature Schnauzer (OR 2,2  $p = 0,003$ ); *purine* in the Dalmatian (OR 352,3  $p < 0,001$ ), the Black Russian Terrier (OR 283,5  $p < 0,001$ ), the Tibetan Spaniel (OR 12,6  $p = 0,001$ ), the Giant Schnauzer (OR 7,0  $p = 0,022$ ), the Bichon Bolognese (OR 6,6  $p < 0,001$ ) and the American Staffordshire Terrier (OR 3,4  $p = 0,042$ ); *cystine* is common in the Basset Hound (OR 40,2  $p < 0,001$ ), the Rottweiler (OR 13,9  $p < 0,001$ ), the French Bulldog (OR 10,4  $p = 0,001$ ), the Wire (OR 7,6  $p = 0,016$ ) and the Short Haired (OR 6,5  $p < 0,001$ ) Dachshund, and in the Chihuahua (OR 4,8  $p = 0,025$ ) breed respectively with high significance.

Under the 12 years of the survey the proportion measured in percentage in the urolith population of Pug (from 0.3 to 4), Bolognese (0.3 to 2), Bichon Frise (0.3 to 1) English Bulldog (0.9 to 2.4) and Yorkshire Terrier (3.8

to 11.1) increased remarkably, while the representation of other breeds like Dalmatians (from 15.6 to 1) , Black Russian Terriers (BRT) (2.1 to 0.4), Poodles (1.8 to 0.4), Basset Hounds (1.2 to 0.4) decreased prominently. The direction of the change in the urolith population was the same as in the whole dog population with some exceptions: there was an opposite change concerning Basset Hound, Chihuahua, Miniature Schnauzer, Pekingese and Puli.

### **3.2 Examination of the prevalence of the genetically determined hyperuricosuria in the dogs with purine urolithiasis**

Between the years 2008 and 2010 a total of 50 non-dalmatian dogs were diagnosed with purine urolithiasis and were invited to the study. Finally blood sample were collected from 24 dogs. 9 dogs of the total 50 were dead or euthanised, the owners of 2 dogs denied the permission of sample collection and in 15 cases the call for the testing remained unanswered. Of the 24 tested dogs in 11 cases the test result were positive for the HUU (7 English Bulldogs, 2 BRTs, 1

Jagd Terrier and 1 crossbreed dog ). In the other 13 cases (4 Yorkshire Terriers, 2 crossbreed dogs, 2 Pekingeses, 2 Bichon Bologneses, 1 Pug, 1 Chihuahua and 1 Dachshund) the test result were negative.

### **3.3 Epidemiology of urolithiasis in cats**

In the study period (from 2006 to 2014) the 77,3 % of the the total of 480 samples were composed of struvit, 17,8 % calcium-oxalate, 1,5 % cystine and 1 % purine.

The mean age of cats affected by struvite urolithiasis was 71,2 months, and 90,1 months in cats with oxalate urolithiasis.

The number of the affected male cats was 2 and 1,5 times greather in struvite ( $n_{str\_male}=241$ ) and oxalate ( $n_{ox\_male}=41$ ) stone disease respectively than the number of feamel cats ( $n_{str\_female}=107$ ,  $n_{ox\_female}=33$ ). The overall ratio of the number of oxalate to struvite stones was 0,22 and showed an increasing pattern from year to year during the study period starting from 0,13 to 0,8. The ratio of the oxalate stones were higher in Persian (0,44) and in British Short Haired cats (1,15). 6 of the total of 7

samples originated from Siamese cats were composed of cystine.

## **4 Discussion**

### **4.1 Epidemiology of canine urolithiasis**

#### **4.1.1 Epidemiologic data**

Epidemiologic data revealed a relatively high and increasing proportion of struvite urolithiasis. The cause of the rise in struvite to calcium oxalate ratio in Hungary unlike other observations is not clear. Previous studies did not really discussed whether the prevalence or the relative number of submissions had changed. As the physical appearance of struvite stones is characteristic in most cases, some practitioners might omit to send the sample for analysis. The lack of submissions of physically well recognisable struvite uroliths may result in apparently decreasing prevalence. Furthermore, prevalence of struvite stones may likely be associated with certain social and economic circumstances which

are probably more expressed in the East-European region as early signs of urinary tract infections remain untreated or mismanaged without expensive laboratory tests and medications.

#### 4.1.2 The affected breeds

The statistical evaluation of the epidemiologic data in the different breeds, compared to the data of the cross breed dogs as reference group, revealed the breeds at risk for a certain type of urolithiasis. These are the breeds where any genetical defect or genetically determined predisposing factor are supposed to play role in the prevalence of urolithiasis. In comparison to other studies our data showed in part differences to other observations.

#### 4.1.3 Struvite urolithiasis

As the result of our study Shih Tzus and English Cocker Spaniels were found to be characterised by high prevalence of struvite urolithiasis as it has been reported

by others. However, our data showed significantly low OR values in some other breeds which were regarded as breeds at high risk for struvite urolithiasis such as Miniature Schnauzer and Dachshund. We observed a completely unique appearance of struvite urolithiasis in Bernese Mountain dogs. The ratio between males and females was 4 to 1 and the mean age of the affected individuals was 33 months (median of 12).

#### 4.1.4 Oxalate urolithiasis

The mean age was the highest among the oxalate stone forming dogs. The fact that the calcium excretion is increasing by age, makes the older age a suspected predisposing factor. Breeds found to be at higher risk are mostly similar to others findings

The breed Shih Tzu was found to be at risk to both oxalate and struvite urolithiasis in several studies except for one paper where this breed was found to be at risk to struvite rather than oxalate urolithiasis. In our study Shih Tzu was at lower risk to oxalate stone formation and at higher risk to struvite formation. Some other breeds that have already been mentioned by others as breeds at risk



such as Chihuahua, Pekingese and English Cocker Spaniel were not found to be predisposed and in addition, even showed expressively low OR values in our study.

#### 4.1.5 Purine urolithiasis

The Dalmatians are known to be affected by a genetically determined condition (HUU) leading to high prevalence of purine urolithiasis. In the current study, beside the Dalmatian breed, the Black Russian Terrier (BRT), English Bulldog, Tibetan Spaniel, Bichon Frise, Bolognese and Yorkshire Terrier were characterised by a significantly high risk of purine urolithiasis compared to crossbreeds. The BRTs affection by this type of urolithiasis has already been reported. In the background a dalmatian-like genetically determined defect was supposed. The predisposition of English Bulldogs and Yorkshire terriers has also been reported elsewhere. English Bulldogs are suspected to be predisposed to HUU and the Yorkshire Terrier breed is affected by inherited porto-systemic shunts that can lead to increased frequency of purine urolith formation.

Concerning the Bolognese dogs there is one paper where the increased prevalence of PSS was concerned as a possible risk factor to purin stone forming. There is no such data on the Tibetan Spaniels.

#### 4.1.6 Cystine urolithiasis

The cystine urolithiasis is caused by the inherited multi-gene defect the cystinuria. The high risk to cystine stone formation in Basset Hounds, Dachshunds, Miniature Pinschers and English Bulldogs has already been reported. Irish Terriers are reported to be highly predisposed to cystinuria. In our study all the 3 samples from Irish Terriers were composed of cystine. The calculated OR value in Irish Terriers compared to crossbreed dogs was very high without statistical significance. The mean age of Irish Terriers and Basset Hounds was 66 and 55 months, respectively. In English Bulldogs the first onset of cystine stones was observed in relatively young individuals. The mean age was 40 months. The known increased frequency of cystine stone disease in French Bulldogs and Rottweilers were proved firstly in our survey by statistical analysis.

Surprisingly, the 2 reportedly most affected breeds (the Newfoundland Dog and the Labrador Retriever) were not represented in our series except for only 1 sample from a Labrador Retriever. Probably in the genetic pool of these breeds in Hungary the allele frequency of this genetic disorder is very low. According to the recently published classification system our results suggest - as male individuals were solely affected - the Type III androgen-dependent cystinuria as the most common form in our dog population.

#### 4.1.7 The incidence of urolithiasis

To our knowledge there is only one paper reporting the prevalence of urolithiasis in dog population in Europe. The calculation was made upon data gathered from a relatively wide time period (26 years). The authors reported 23 to 24 cases/10,000 dogs within this period. It meant an average incidence rate of 0.88 to 0.92/10,000/year. Our calculation revealed approximately two times higher incidence indicating that yearly at least one dog potentially affected by urolithiasis should be expected amongst 5,600 individuals.

#### 4.1.8 Change of the prevalence of the mostly affected breeds and its effect on the occurrence of urolithiasis

The proportion of the most affected breeds in the whole population increased around 5 times concerning Yorkshire Terrier, West Highland White Terrier, Havanese, Bichon Frise, Beagle, Chihuahua, English Bulldog and the Pug throughout the study period. Yorkshire Terriers and English Bulldogs were characterised by calcium oxalate in combination with purine and purine with cystine urolithiasis, respectively. The increasing presence of these breeds adumbrates the increasing prevalence of calcium oxalate, purine and cystine urolithiasis. In addition the expressed diminution of the proportion of the breeds Dalmatian, German Shepherd and Dachshund which were characterised by purine, struvite and cystine urolithiasis respectively, was detected in the population.

The change in breed proportions in the urolith database showed more or less the same evolution as in the whole population. As regard to breeds at risk the prevalence of

Yorkshire Terriers increased while the proportion of Dalmatians and the Black Russian Terriers decreased in a large scale during the study period among the stone forming dogs.

Whilst no sample was registered from the Havanese breed in the first year of the survey, 22 were submitted in the last two years leading to an increase in the proportion. The same change was detected in the whole population too. Individuals of small breeds are exposed to environmental factors that can promote oxalate stone formation. The increasing prevalence of Pug, Havanese, Chihuahua, Bichon Frise were also observed in the population. The observed trend predicts the plausible increasing pattern of oxalate urolithiasis and the decreasing nature of struvite, purine and cystine urolithiasis.

While the proportion decreased in the population the prevalence consequently increased in the urolith database concerning Pekingese, Miniature Schnauzer and Puli. Pekingese and Miniature Schnauzers are associated with promoting factors to urolithiasis. The increasing expression of these factors is presumed in the background, but it remained unknown whether it was due to certain genetic shift or environmental impact.

Concerning the breed Puli this alteration cannot be explained.

The opposite change was observed in Basset Hounds and Chihuahuas. Basset Hounds are strongly susceptible to genetically determined cystinuria. Genetic shift in the gene pool, decreasing frequency of the mutated gene(s) in this breed in Hungary can result in this alteration. This change concerning the Chihuahuas cannot be explained by these or any other known facts.

#### **4.2 Examination of the genetic background of the purine urolithiasis in dogs**

Nine of 50 dog died between the one year period from the diagnose and blood sample collecting. The 18 % mortality between the dogs diagnosed by purine urolithiasis regardless the underlying factor means a relatively poor prognostic value. Among the dogs died before the sample collection there was none of breed known to be affected by HUU.

However the genetic defect has been detected in every individual affected by purine urolithiasis from breeds known to be predisposed to HUU.

The Yorkshire Terriers are predisposed to inherited PSS leading to increased frequency of purine urolithiasis. In the breed Pekingese the increased prevalence of purine stones also has been mentioned by others but the underlying factor is still unknown. Based on our findings it is unlikely the HUU. As there was 2 positive dog from previously not affected breeds (a Jagd Terrier and a crossbreed one) the presence of the SLC2A9 gene mutation can be higher than it is expected by the prevalence of purine stone disease or there are other currently unknown predisposing factor too beside the genetic alteration. The presence of unknown predisposing factor is suspected in the background of the purine urolithiasis of one affected BRT in which the genetic test showed heterozygous positive result. In a previous study the uric acid to creatinin ratio in the urine of heterozygous dogs was same as in normal dogs. The clinical examination of this heterozygous 4 years old BRT dog yielded normal results. Based on these findings it has to concern that heterozygous dogs are also at risk for purine stone formation. Further investigations needed for the identification of the plausible unknown predisposing factor.

### **4.3 Epidemiology of urolithiasis in cats**

In our series the proportion of cats affected by struvite stones are higher than in other studies especially in the US, Canada, Switzerland and Europe and the ratio of oxalate to struvite stones showed an increasing pattern.

In the background of this phenomenon the change in the composition of commercial cat foods is suspected.

As the same process was observed in other studies in the past decade an opposite change – the increasing ratio of struvite stones – was detected in some studies especially in the US.

According to others findings oxalate stones were observed in cats of older ages.

In our survey Persian and British Short Haired cats were found to be affected more frequently with oxalate stone disease. Concerning the linkage between the breed and the stone diseases conflicting data are published in different studies.

In Siamese cats according to others findings high prevalence of cystine stone disease was observed. The pattern of cystine disease is different than those in dogs. More female than male individual were affected. This



findings is surprising because in the background of cystine stone disease the same genetic condition is suspected in both species.

## 5 New scientific results

1. We described first the epidemiology of canine urolithiasis in Hungary.
2. We considered first the incidence of urolithiasis in dogs.
3. We proved the presence of predisposing factors in several breeds.
4. Predisposition was observed in some other previously not reported breeds:
  - a. Black Russian Terrier – purine urolithiasis
  - b. Tibetan Spaniel, Bolognese – purine urolithiasis
5. Our findings statistically proved the predisposition in some other previously observed breeds too:
  - a. German Shephard dog – struvite
  - b. Bernese Mountain dog – struvite affecting mainly young males
  - c. French Bulldog, Rottweiler – cystine urolithiasis
6. In the study we described the change in the distribution of breeds in the population during the study period. The correlations between the

change in the population and the change in the dogs affected by stone diseases were also evaluated.

7. Our examinations proved the presence of the genetically determined HUU in the background of purine stone formation in the Black Russian Terrier and the English Bulldog.
8. We described first the epidemiology of urolithiasis in cats in Hungary.

## **6 Scientific publications of the thesis**

1, **Bende, B.**, Dunay, M., Zsömböly, M. (2001): Cystine urolithiasis in dogs. Hun. Vet. J. 123, 486-491. **IF: 0,184**

2, **Bende, B.**, Szabó, O., Reiczigel, J. (2003): The dog population of Budapest at the millennium. Hun. Vet. J. 125, 340-345. **IF: 0,089**

3, **Bende, B.**, Németh, T. (2004): High prevalence of urate urolithiosis in the Russian black terrier. Vet. Rec. 155, 239-240. **IF: 1,147**

4, Del Angel Caraza, J., Pérez-Garcia, C.C., **Bende, B.**, Díez-Prieto, I., Garcia-Rodriguez, B. (2011): Mouse barley awn (*Hordeum murinum*) migration induced cystolithiasis in 2 male dogs. Can. Vet. J. 52, 67-69. **IF: 1,063**

5, **Bende, B.**, Németh, T. (2015): Epidemiology of urolithiasis in cats in Hungary from 2006 to 2014 (480 cases). Hun. Vet. J. 137, 305-313

6, **Bende, B.**, Kovács, K. B., Solymosi, N., Németh, T.  
(2015): Characteristics of urolithiasis in the dog  
population of Hungary from 2001 to 2012. *Acta Vet. Hun.*  
63, 323-363

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