Assessment of urinary cystine concentration in dogs in the Czech Republic

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Abstract

The prevalence of cystinuria as an inborn error of metabolism in dogs is unknown. The purpose of this study was to evaluate the prevalence of cystinuria in dogs of various breeds in the Czech Republic. In total, 326 voided urine samples from client owned dogs were obtained. Samples were divided into four groups according to the breed - Irish Terriers (n = 58), Dachshunds (n = 67), French Bulldogs (n = 64) and a group of various breeds (n = 137). Urinary cystine concentration was measured using liquid chromatography/mass spectrometry, urinary creatinine concentration was determined by Jaffe method. Samples with urinary cystine concentrations above 178 µmol/g creatinine were considered as cystinuric. Urinary cystine concentration above the upper limit was found in 71 dogs (21.8%) with significantly higher incidence in intact males of Irish Terriers. In general, cystinuria was more common in intact males. In Irish Terriers, Dachshunds, French Bulldogs, German Shorthaired Pointer, Pomeranian, and Fox Terrier, cystinuria was identified in females. Cystinuria appears to be a relatively common finding in dogs in the Czech Republic, with some breeds being more affected. The finding of cystinuria in two female Irish Terriers calls into question the classification of cystinuria in this breed as androgen dependent only. The detection of cystinuria in related dogs suggests that other individuals in the same family should be examined as part of the management of a cystinuric patient.

Irish Terriers, Dachshunds, French Bulldogs, urolithiasis, tubulopathy, aminoaciduria

Cystinuria is an inborn metabolic disorder characterized by abnormal intestinal and renal transport of cystine and the other dibasic amino acids ornithine, lysine, and arginine (COLA [cystine, ornithine, lysine, arginine]). Because of the defect, excessive amounts of these amino acids are excreted in urine (Mattoo and Goldfarb 2008). At normal urine pH (5–7), cystine is relatively insoluble and it may lead to crystal precipitation and urolith formation typically in the lower urinary tract (reviewed by Kovaříková et al. 2021). Subsequent urolithiasis may be associated with typical clinical signs such as dysuria, haematuria or anuria in case of urethral obstruction. Nevertheless, only cystinuria may be associated with clinical consequences because the other dibasic amino acids are relatively soluble in urine (Mattoo and Goldfarb 2008). Clinical symptoms (related to protein malnutrition, COLA deficiency) are not reported in cystinuric patients without urolithiasis (Bartges and Callens 2015).

Cystine urolithiasis is diagnosed almost exclusively in male dogs, typically in young adult intact males. Among the canine breeds which are frequently mentioned in association with cystine urolithiasis are English Bulldogs, Newfoundland dogs, Dachshunds, Chihuahuas, Staffordshire Bull Terriers, Rottweilers, French Bulldogs, and Miniature Pinschers (Kovaříková et al. 2021). According to Lulich and Ulrich (2010), cystine urolithiasis has been diagnosed in more than 170 canine breeds. The prevalence of cystine urolithiasis differs with geographic region. Historically, the prevalence is much higher in European

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countries in comparison with the United States and Canada as reviewed by Kovaříková et al. (2021). The most recent studies report a prevalence of up to 15% in Europe but only 3% in United States. The difference between the results from North America and Europe may be explained by the proportion of dogs with androgen-dependent type of cystinuria. In Europe, neutering of male dogs is not as common as in North America, so there is a higher number of intact males that can develop androgen-dependent cystinuria. Nevertheless, increasing occurrence is reported in Europe as well as North America. Cystinuria may be diagnosed by the finding of cystine urolithiasis, detection of cystine crystals in urine, measurement of urinary amino acid concentration (cystine alone or together with the other dibasic amino acids ornithine, lysine, and arginine in COLA test) or by genetic tests, in some breeds (Kovaříková et al. 2021).

The purpose of this study was to evaluate the urinary cystine concentration in selected breeds and the group of various breeds in the Czech Republic.

Materials and Methods

Animals and urine sampling

Dachshunds and French Bulldogs are regularly mentioned as breeds affected by cystinuria and they are also very popular breeds in the Czech Republic. Therefore, we decided to attempt to assess the situation around cystinuria in these breeds. The third group was made of Irish Terriers. Although it is not one of the most affected breeds, breeders report that cystinuria is a growing problem in the Czech population of Irish Terriers. Representatives of different breeds were included in the fourth group to evaluate the normal dog population.

Urine samples were collected between January 2020 and June 2021. The study was announced through social networks and the owners were recruited mainly among the staff of the University of Veterinary Sciences Brno, Czech Republic. The breeders of selected breeds were contacted through Czech kennel clubs. In total, 326 dogs were enrolled in the study by volunteering owners or breeders. There were 157 males (24 of them were neutered) and 169 females (57 of them spayed). The mean age was 5.6 years, ranging from 3 months to 17 years. Four groups were created according to the breed of dogs: a group of Irish Terriers (n = 58), a group of Dachshunds (n = 67), a group of French Bulldogs (n = 64) and a group of various other breeds (n = 137). All groups are presented in Table 1; dog breeds included in the fourth group are listed in Table 2. Dogs without a pedigree were classified as crossbreeds.

A brief medical history was taken, dogs with previously diagnosed cystine urolithiasis or cystine crystalluria were not included in the study. As a part of history, owners were asked about their dog's diet, however, the exact protein content was not recorded. Urine was routinely collected by the owners during spontaneous voiding and placed into a plastic syringe or a plastic tube.

After the sampling, urine specific gravity was measured in all samples via refractometry with a hand refractometer (RUR2-ATC, Bellingham + Stanley, Tunbridge Wells, UK). Chemical characteristics of the samples (pH, protein, glucose, ketone bodies, bilirubin, blood) were assessed using urinary dipsticks (HeptaPHAN, Erba Lachema, Czech Republic) (data not shown). Urine samples were stored at −20 °C and examined within 2 months. The reported stability of cystine and creatinine in frozen urine samples is 70 days and 3 months, respectively (Mayo Clinic Laboratories; Rossi et al. 2012).

Analysis of urinary cystine concentration

For each sample, volumes of 50, 100, and 300 µl were added into 3 glass test tubes. Each test tube was then spiked with 100 µl of internal standard solution (isotopically labelled cystin-$C^{13}{}_2$, 50 mg/l in deionised water) and diluted into a final volume of 2 ml with deionised water. One millilitre of diluted and spiked sample solution was passed through a preconditioned cartridge (1 ml methanol and 1 ml water). The cartridge was then allowed to dry for 10 min, and the analyte was eluted with methanol (1 ml) into the glass test tube (10 ml). Eluted solution was evaporated until dryness under gentle nitrogen stream (at 40 °C). In order to perform derivatization,

Table 1. Characteristics of groups created according to the breed of dogs. Age is expressed as mean ± standard deviation. Age range is given in brackets.

Group	Irish Terriers	Dachshunds	French Bulldogs	Various breeds
Number	58	67	64	137
Sex (m/f)	32/26	29/38	26/38	70/67
Neutered/spayed	3/4	4/15	6/9	11/29
Age	4.3 ± 2.9	7.1 ± 4.4	4.3 ± 2.9	6.1 ± 4.0
	(3 months–14 years)	(1–17 years)	(4 months–12 years)	(6 months–15 years)

Table 2. List of other canine breeds assessed in the group of various breeds.

Breeds	Number
Crossbreed	22
Border Collie	8
Czechoslovakian Wolfdog, German Shepherd, Golden Retriever	5
Belgian Shepherd, Labrador Retriever	4
Australian Shepherd, German Shorthaired Pointer, Jack Russel Terrier, Miniature Schnauzer, Sheltie, Whippet, Weimaraner, Yorkshire Terrier	3
Border Terrier, Chesapeake Bay Retriever, Giant Schnauzer, Greyhound, Parson Russel Terrier, Pomeranian, Siberian Husky, Small Münsterlander, Smooth Collie, Standard Poodle, Standard Schnauzer	2
Airedale Terrier, Alaska Husky, Australian Cattle Dog, Bernese Mountain Dog, Bohemian Shepherd, Borzoi, Brittany, Chihuahua, Coton de Tulear, Dalmatian, Dobermann, English Cocker Spaniel, English Springer Spaniel, Entlebucher Mountain Dog, Eurohound, Flat-coated Retriever, Fox Terrier, Greater Swiss Mountain Dog, Hovawart, Italian Greyhound, Maltese Dog, Miniature Poodle, Newfoundland Dog, Nova Scotia Duck Tolling Retriever, Papillon, Pitbull Terrier, Podenco, Portuguese Water Dog, Pug, Rhodesian Ridgeback, Rottweiler, Rough Collie, Saluki, Samoyed, Toy Poodle, Vizsla, Welsh Corgi, West Highland White Terrier	1

a volume of 300 µl of sodium bicarbonate buffer (100 mM; pH = 8.9) and 300 µl of dansyl chloride solution in acetone (1 g/l) were added into dry residues in glass test tube, gently vortexed and incubated at 70 °C for 30 min. Subsequently, the mixture was evaporated until dryness under gentle nitrogen stream (at 40 °C) and dry residues were reconstituted in 1 000 µl of methanol filtered through a 0.2 mm nylon filter (Millipore, Billerica, MA, USA) and used for liquid chromatography/mass spectrometry (LC/MS) analysis.

The Thermo Scientific UHPLC Accela 1250 system was connected to the Thermo Scientific TSQ Quantum Access MAX Triple Quadrupole Instrument (Thermo, San Jose, USA) equipped with a heated electrospray ionization probe. A Titan C_{18} (2.1 mm × 100 mm, 1.9 µm; Supelco, Bellefonte, PA, USA) column was used at a constant flow rate of 250 µl/min. The mobile phase consisted of 5 mM ammonium formate in water (adjusted to pH = 8.9 by a 10% solution of aqueous ammonia; solvent A) and methanol (solvent B). The gradient used was: 0–2.0 min linear gradient from 20 to 95% B; 2.0–8.0 min held at 95% B; 8.0–8.4 min from 95 to 20% B and 8.4–9.0 min held at 20% B in order for the column to re-equilibrate before the next injection. The full loop injection volume of the sample was set at 10 µl. The heated electrospray ionization was operated in the positive mode under the following conditions: capillary temperature, 350 °C; vaporizer temperature, 350 °C; sheath gas pressure, 35 psi; auxiliary (drying) gas, 10 au; and spray voltage, 3 300 V.

For our quality assurance and quality control program, the instrument was calibrated daily with multi-level calibration curves. Procedural blank and solvent blank were analysed for every set of 10 samples. To evaluate the method's process efficiency (PE), extraction recovery (RE), and matrix effect (ME), ten samples and ten sample extracts were spiked with standard solution at target levels 80 mg/l. The matrix effect was calculated as ME (%) = (B/A – 1) × 100, where A is the peak area of the standard solution and B is the peak area of the post extraction standard addition. The extraction recovery was calculated as RE (%) = C/B × 100, where C is the peak area of the pre-extraction standard addition and the process efficiency was calculated as PE (%) = C/A × 100. The method process efficiency was 68.8%; extraction recovery was 79.7% and the matrix effect was −13.6%. The inter-day precision expressed as a relative standard deviation was 8.6% and the inter-day accuracy was −5.1%. The limit of detection determined as 3:1 signal versus noise value was 0.036 mg/l. The lower limit of quantification was defined as the lowest concentration level of calibration curve (0.2 mg/l) with precision and accuracy less than 20% and the corresponding signal/noise ratio greater than 10.

Standards of cystine and isotopically labelled cystin-C^{13}_2 (internal standard), sodium bicarbonate, sodium hydroxide, dansyl chloride, formic acid, ammonium formate and solution of aqueous ammonia (25%) were purchased from Sigma-Aldrich (St. Louis, USA). Acetonitrile, methanol and acetone were purchased from Chromservis s.r.o. (Prague, Czech Republic) and were LC/MS purity (≥ 99.9%). Nylon syringe filters were purchased from Millipore (USA). Oasis WAX cartridges (3 ml, 60 mg, 30 µm) were purchased from Waters (Milford, MA, USA).

Analysis of urinary creatinine concentration

Analysis of urinary creatinine was performed spectrophotometrically by Jaffé method using the biochemical analyser Konelab 20i (Thermo Fisher Scientific, USA) and a commercial kit (Biovendor, Czech Republic). Samples were centrifuged (3 min, 800 × *g*) and the diluted supernatant (i.e. 50 µl of urine sample + 2 450 µl of ultrapure water) was used for analysis.

Statistical analysis

The concentration of urinary cystine was expressed as μmol/g creatinine. Dogs with urinary cystine concentrations above the 179 μmol/g creatinine are referred to as cystinuric (Brons et al. 2013). Statistical analysis was carried out using statistical software Unistat for Excel 6.5. (Czech Republic) and Statistica 8.0 for Windows (StatSoft, USA). Chi-square (χ^2) test of independence was used to determine if there was a significant difference between frequencies of dogs in individual groups included in our study (female × male, breeds, neutered × intact). A significant difference was considered when $P < 0.05$. Data of cystine concentrations in text are presented as means ± standard deviation (SD).

Results

In 71 dogs (21.8%), urinary cystine concentration was above the upper limit (607 ± 395 μmol/g creatinine; range 184–2 260 μmol/g creatinine). In the remaining 255 dogs (78.2%), the urinary cystine concentration was below or equal to 178 μmol/g creatinine (63.1 ± 33.6 μmol/g creatinine; range 6–178 μmol/g creatinine). The distribution of urinary cystine concentrations for the different breeds and for the group of various breeds is presented in Fig.1. The distribution of values below or equal to 178 μmol/g creatinine in all non-cystinuric dogs is presented in Fig. 2. Details of urinary cystine concentration (mean, standard deviation, range) for each group are presented in Table 3, characteristics of cystinuric dogs in the group of various breeds are outlined in Table 4. The highest prevalence of cystinuric dogs in all breeds was found in males, namely intact dogs. The highest frequency was demonstrated in Irish Terriers, with 75.9% of the intact males assessed as cystinuric (Fig. 3). When comparing the frequency of cystinuria in neutered males of the breeds studied, no significant differences were observed between the categories. Similar was the case for neutered and intact females. However, when comparing the frequency of cystinuria in intact males, significant differences were found between Irish Terriers and all other groups ($P < 0.05$).

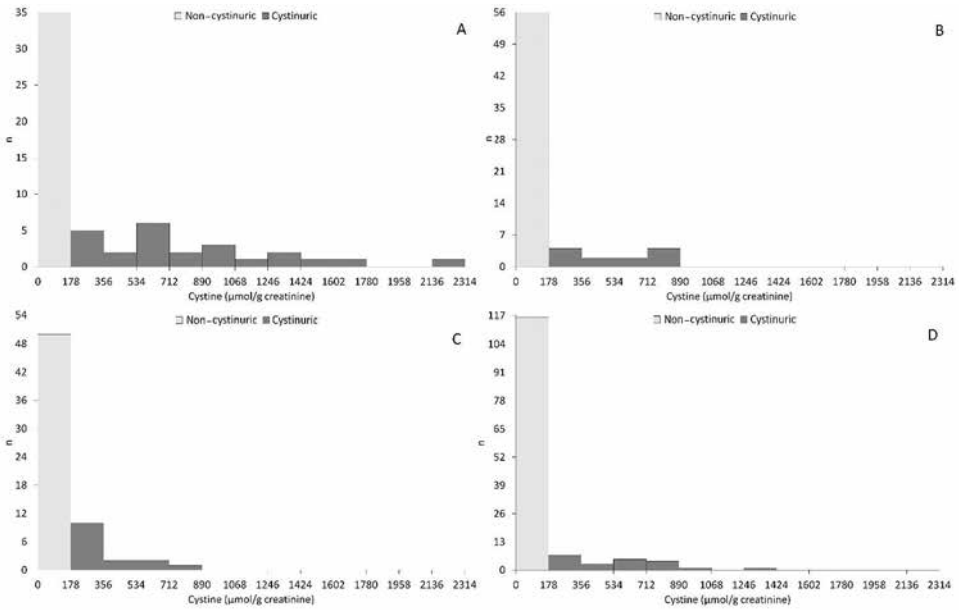

Fig. 1. Frequency distribution of urinary cystine concentration according to the breed of dogs. A – Irish Terriers; B – Dachshunds; C – French Bulldogs; D – Various breeds.

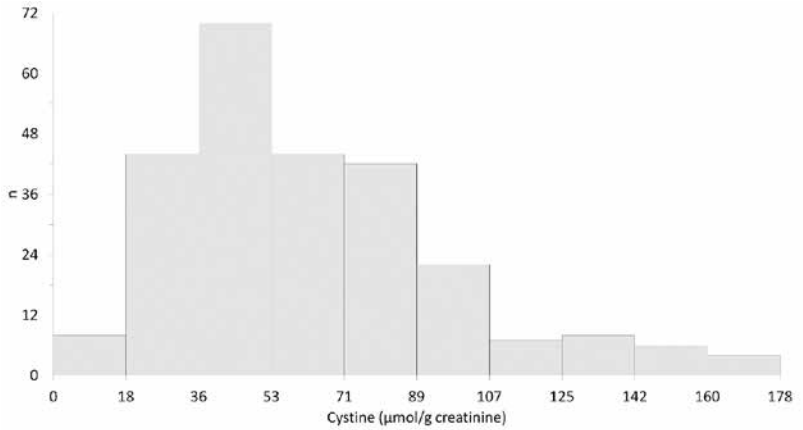

Fig. 2. Frequency distribution of urinary cystine concentration for non-cystinuric dogs.

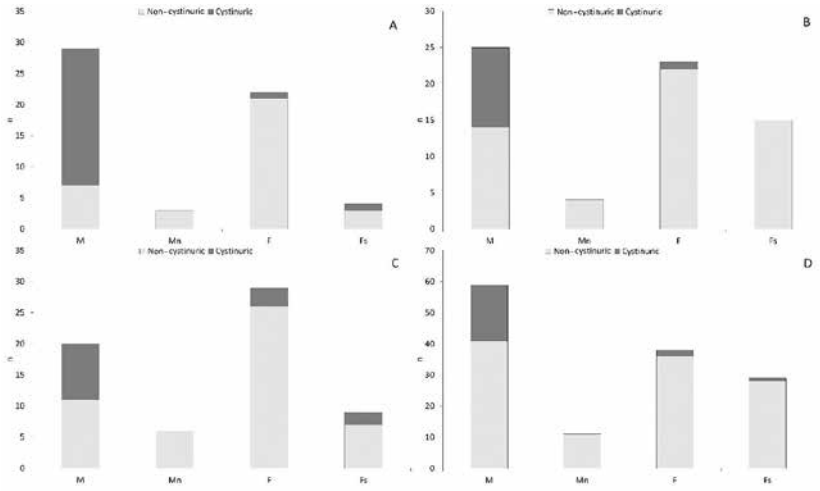

Fig. 3. Proportion of non-cystinuric and cystinuric dogs according to the breed of dogs and sex. A – Irish Terriers; B – Dachshunds; C – French Bulldogs; D – various breeds. M – males; Mn – males neutered; F – females; Fs – females spayed.

Table 3. Urinary cystine concentrations (µmol/g creatinine) in all four studied groups of dogs; comparison of cystinuric and non-cystinuric dogs.

Group	Irish Terriers	Dachshunds	French Bulldogs	Various breeds
Cystinuric				
Number	24	12	14	21
Mean ± SD	823 ± 524	500 ± 229	395 ± 204	563 ± 283
Range	188–2260	184–808	184–826	226–1320
Non-cystinuric				
Number	34	55	50	116
Mean ± SD	71.1 ± 34.8	80.0 ± 37.3	65.6 ± 35.1	51.7 ± 26.1
Range	14.7–159	16.0–173	16.9–178	6.12–161

Table 4. Characteristics of cystinuric dogs in the group of various breeds.

Breed	Number of cystinuric dogs (total number of dogs in this breed)	Signalment
Crossbreed	2 (22)	Crossbreed looking like a Dachshund, intact male, 7 y; Crossbreed looking like Belgian Shepherd, intact male, 9 y
German Shorthaired Pointer	2 (3)	Intact male, 4.5 y; Spayed female, 2 y
Pomeranian	2 (2)	Intact male, 13.5 y; Intact female, 3.5 y
Sheltie	2 (3)	Intact male, 1 y; Intact male, 8 y
Small Münsterlander	2 (2)	Intact male, 6 y; intact male, 7 y
Australian Shepherd	1 (4)	Intact male, 8 y
Border Collie	1 (8)	Intact male, 3 y
Chesapeake Bay Retriever	1 (2)	Intact male, 8 y
Fox Terrier	1 (1)	Intact female, 13 y
Italian Greyhound	1 (1)	Intact male, 3 y
Jack Russel Terrier	1 (3)	Intact male, 10 y
Saluki	1 (1)	Intact male, 6 y
Standard Poodle	1 (2)	Intact male, 1.5 y
Toy Poodle	1 (1)	Intact male, 11 y
Welsh Corgi	1 (1)	Intact male, 4 y
Whippet	1 (3)	Intact male, 5 y

y – years of age

Discussion

According to the latest reports, the prevalence of cystine urolithiasis in dogs in the Czech Republic is up to 16% with a steady upward trend (Kučera and Kořistková 2017). In the group of cystinuric dogs diagnosed during the period 2003–2016 in the Czech Republic, the most often affected breed were Dachshunds (21.3% of all cystinuric dogs) and French Bulldogs which are considered among the six most affected breeds (Kučera and Kořistková 2018).

In total, 71 dogs in our study (21.8%) were identified as cystinuric. This high number is affected by high prevalence of cystinuria in Irish Terriers. In the group of various breeds, increased concentration of urinary cystine was found in 21 dogs (15.1%). In the Czech Republic, the breeds most frequently affected by cystine urolithiasis dogs are Dachshunds, English Bulldogs, Chihuahuas, Yorkshire Terriers, Staffordshire Bull Terriers and French Bulldogs (Kučera and Kořistková 2018). In our study, Dachshunds and French Bulldogs have their own groups. Of the remaining at-risk breeds, four dogs (three Yorkshire Terriers - two males, one of them castrated, and a spayed female; one Chihuahua - intact female) were in the group of various breeds and none of them were found to have elevated urinary cystine concentrations. Thus, the high incidence of cystinuria found in this group is not due to intentional selection of at-risk breeds but rather by the actual incidence of cystinuria in the Czech canine population.

The results of our study suggest that cystinuria in dogs is relatively common. Irish Terriers seem to be affected to a greater extent, although the results of monitoring cystine urolithiasis in different breeds of dogs in the Czech Republic do not corroborate this finding. The fact that Irish Terriers do not feature prominently in the ranking of breeds diagnosed with cystine urolithiasis may be influenced by the fact that the Irish Terrier

population is much smaller than the population of Dachshunds and French Bulldogs bred in the Czech Republic. According to data provided by kennel clubs, an average of 70 dogs of this breed are being added to the population in the Czech Republic each year. Dachshunds and French Bulldogs are much more popular, with an average of 600 French Bulldogs and approximately 2 000 Dachshunds entered into the studbook each year. Although the prevalence of cystinuria was lower in Dachshunds and French Bulldogs in our study, the absolute numbers of dogs affected by cystine urolithiasis are higher.

Furthermore, not all dogs with cystinuria will develop cystine urolithiasis, and cystinuria is considered to be a predisposing rather than a primary causative factor (Brand et al. 1940). In general, formation of uroliths depends on the concentration of crystallogenic compounds in urine which was recognized as the primary factor in the precipitation and subsequent growth of calculi. Nevertheless, other factors may contribute to urolithiasis – urine pH, the presence or absence of promoters or inhibitors of urolithiasis and to some degree the amount of time urine is stored in the bladder (Bartges and Callens 2015). In this study, we did not differentiate among the types of Dachshunds because then there would not be a sufficient number of individuals for statistical evaluation. Moreover, the available reports on the prevalence of cystinuria also do not distinguish different types of Dachshunds.

In general, male dogs are affected by cystine urolithiasis significantly more often than females (98.8% vs 1.2%) (Kovaříková et al. 2021). Two theories have been hypothesized. The first says that males are more affected because of the type III – androgen-dependent type of cystinuria, which is reported in Mastiffs and related breeds, Scottish Deerhounds, and Irish Terriers, and is suspected in many other canine breeds (Brons et al. 2013). The second is that females are also affected by cystinuria and may form uroliths as shown in Newfoundland dogs, but do not have clinical symptoms because of the anatomic difference between the male and female urinary tract, with small stones passing through the short and wide female urethra (Casal et al. 1995). Our study shows that even though females of various breeds are also affected by cystinuria, affected males predominate (60 males out of 71 cystinuric dogs, 84.5%).

In Irish Terriers, cystinuria is a well-known condition. To the authors' knowledge, the first report on cystinuria in male Irish Terriers was published in 1936 (Green et al. 1936). Later, the family history of the first case and its affected relative were described (Brand et al. 1940). In 2011, Giger et al. (2011) conducted a large study on 222 Irish Terriers from Europe and Australia, where urinary amino acid concentrations were determined by COLA test and sequencing of the coding regions of the SLC3A and SLC7A9 genes was performed in EDTA blood samples. Clinically healthy dogs and dogs with cystine urolithiasis were enrolled in the study. All females had normal urinary cystine concentrations (less than 150 μmol/g creatinine). Positive COLA test was noted in 51 intact males (of which 10 males formed cystine calculi). When the percentage rate is calculated, 36.7% of males were affected. Unfortunately, in this work, the numbers of intact and castrated males were not reported. They did not identify any mutation using DNA analysis. Giger et al. (2011) named this condition in Irish Terriers as a non-type I cystinuria which occurs only in males. According to their results, castration lowers the concentration of cystine and the other dibasic amino acids and castration appears to have a greater effect on urinary amino acid concentrations in comparison with a therapeutic diet. Cystinuria in Irish Terriers was later classified as type III (androgen-dependent) (Brons et al. 2013). The group of Irish Terriers in our study consisted of 32 males (three of them were castrated) and 26 females (four of them spayed). In the group of Irish Terrier males, 22 (68.8%) of them were identified as cystinuric. This number is much higher than the calculated percentage rate of 36.7% of affected males reported by Giger et al. (2011). The percentage of cystinuric dogs may be even higher in our study. Two males were sampled at the age of three months with negative

results (urinary cystine concentration of 99 and 83 μmol/g creatinine, respectively). Nevertheless, at this age, it is too early for a definitive decision as to whether a dog is cystinuric or not.

As cystinuria in Irish Terriers has been classified as androgen-dependent, it is very surprising that an increased urinary cystine concentration was found in two females: one an intact 2-year-old, and the other a spayed 6-year-old, with urinary cystine concentrations of 1 100 μmol/g creatinine and 683 μmol/g creatinine, respectively. The owners of these two females were very cooperative and allowed us to obtain more information about the dogs' pedigrees. The older female was the mother of the younger one as well as the mother of another (intact) male with increased urinary cystine concentration in this study. The offspring were from two different litters but from the same parents. Thus, this family lineage deserves closer monitoring of urinary cystine concentrations along with an assessment of their pedigree. Nevertheless, the finding of cystinuria in two Irish Terrier females suggests the presence of another type of cystinuria in this breed as well.

French Bulldogs are often mentioned in association with cystine urolithiasis (Kovaříková et al. 2021). Giger et al. (2011) classifies cystinuria in French Bulldogs as type III (androgen dependent), but our results do not support it. We found also increased urinary cystine levels in five females (three intact and two spayed) of French Bulldogs from a total of 38 (13.2%). For further evaluation of whether the androgen-dependent type of cystinuria occurs in French Bulldogs it would be useful to examine samples of cystinuric dogs before and after neutering and to assess any decrease in urinary cystine concentration. However, we did not include cystinuric dogs in our study. No difference was found when comparing the urinary cystine concentration of cystinuric and non-cystinuric dogs.

Dachshunds are a very popular breed and they are also frequently reported in association with cystine urolithiasis (Kovaříková et al. 2021). In the Czech Republic, Dachshunds account for more than one fifth of all cases of cystine urolithiasis (Kučera and Kořistková 2018). Although the group of cystinuric Dachshunds was dominated by intact males, an elevated urinary cystine concentration was also found in one intact female. So the occurrence of androgen dependent cystinuria in Dachshunds is rather unlikely. On the other hand, Cruciani et al. (2020) described cystine urolithiasis in an intact male Dachshund treated by percutaneous cystolithotomy and immediate neutering. This dog showed no clinical problems more than a year after the procedure, which would indicate the presence of type III cystinuria. However, in this case, the concentration of urinary cystine was not measured, so it is uncertain whether there was a decrease after neutering. Moreover, the period without clinical symptoms was relatively short.

Cystinuria was detected in two intact Small Münsterlander males aged 6 and 7 years. They were related, the younger dog being a direct descendant of the older one. According to the owner, they never had urinary problems. Elevated urinary cystine concentrations were also found in two Pomeranians (an intact male aged 13.5 years and an intact female aged 3.5 years). Again, these dogs were related. The female was a second generation direct descendant of the older dog. The finding of cystinuria in related dogs suggests that there could be other affected individuals in the family. Thus, when managing cystinuric patients, it would also be advisable to measure urinary cystine concentration in other related dogs. Thanks to this, the affected dogs could be detected before their possible inclusion in breeding. At the same time, it could provide further information on the mode of inheritance. However, our results suggest that several different mutations causing cystinuria could occur in a single breed.

The specific mutation causing cystinuria and the mode of inheritance are known in only a few canine breeds (Brons et al. 2013). In Newfoundland dogs, the mutation has been known for the longest time (Casal et al. 1995). The possibility of identifying not only affected dogs but also the carriers may have an impact on breeding programmes allowing

a reduction of cystinuria in the future. In our study, cystinuria was found in two unrelated German Shorthaired Pointers out of three and similarly in two unrelated Shelties out of three that were examined. Although this is not a representative sample, attention could be paid to these breeds in the future with regard to cystinuria.

The androgen dependent type of cystinuria, in which castration has a therapeutic effect, is important in terms of treatment of cystinuria. Due to the overwhelming prevalence of males affected by cystine urolithiasis, this type is assumed in many other canine breeds. Our study showed that even in breeds where androgen dependent type of cystinuria is described, females can also be affected, so either it is a different type of cystinuria or there are several types of cystinuria in one breed. Thus, measurement of the urinary cystine concentration at the time of diagnosis and three months after castration can be recommended for obtaining further information.

A limitation of this study is the selection of dogs which does not provide a representative sample of the population. The decision to provide a urine sample was entirely up to the owners. This certainly affected the Irish Terrier group, where we were often faced with unwillingness to provide a urine sample for analysis or information about the dog's pedigree.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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