

Toward a Better Breed—The Deerhound Cystinuria Study

Last year I wrote about a potentially life-threatening inherited disease called cystinuria, in which the amino acid cystine gets into the urine where it doesn't belong. Cystinuria occurs in many breeds and has been studied extensively in Newfoundlands and Mastiffs, but I knew of few Deerhound cases. I suggested that breeders ought to have a urine sample tested for cystine before using a hound for breeding, but I believed the disease was uncommon in Deerhounds and expected that urine test results would rarely be positive.

At least one breeder took my suggestion about testing breeding stock, and the resulting cascade of events has put cystinuria into the forefront of Deerhound health issues. It's also brought us into partnership with the same research team that identified the gene responsible for cystinuria in Newfoundlands. Thanks especially to Joan Creel's leadership, we're collaborating with a University of Pennsylvania research team to study cystinuria in Deerhounds and identify the responsible gene. To do so will require many people's help.

Background—What is Cystinuria?

Urine produced in the kidneys flows into the urinary bladder, which lies just in front of the pelvis. The bladder periodically empties itself by expelling urine through a narrow tube called the urethra that runs back through the pelvis. In females, the urethra is short and opens just inside the vulva. In males, it's long and curves around under the skin surface below the anus to run forward to open at the tip of the penis. Besides being longer and less straight than the female urethra, the male urethra is relatively smaller in diameter.

The kidneys make urine by removing plasma from the bloodstream and then re-absorbing potentially useful substances out of it and putting them right back into the bloodstream. What's left behind is urine. Among the useful substances normally retained by the kidneys are cystine and other amino acids, which are the building blocks that cells use to make proteins of all sorts.

In dogs with cystinuria, the kidneys don't adequately re-absorb cystine, so it shows up in the urine. Until recently, veterinarians were taught that cystine was always present in the urine of dogs with cystinuria, but that turns out not to be so. Urine from affected Newfoundlands always contains cystine, but Mastiffs with cystinuria sometimes have cystine-free urine. How this happens is unclear and under study.

The kidneys do one other thing to the urine that has important consequences for dogs with cystinuria—they re-absorb water out of the urine as they make it. As a

result, the concentration of a given substance in urine ends up to be greater than its concentration in blood. If the concentration of a substance in urine exceeds its maximum solubility, then the substance will form crystals that drop out of solution. You can get the same result by trying to dissolve too much sugar in your iced tea.

As it happens, cystine is poorly soluble in urine—especially urine that's acidic like dog urine—so it tends to form crystals if it sits in the bladder for a while. These crystals can continue to grow until they become quite large and are called uroliths. Uroliths can harm dogs in two ways—first, they can irritate the delicate bladder lining and predispose a dog to urinary tract infections, and second, they can lodge in the urethra and prevent a dog from urinating at all. Urinary obstruction is a life-threatening emergency. Surgery almost always is required to remove the obstructing urolith.

Cystinuria typically is diagnosed only when uroliths form and make a dog ill. Because small cystine uroliths can pass unnoticed through the larger diameter urethras of females, bitches often go undiagnosed. But even small uroliths can lodge in the narrower diameter urethras of males, calling immediate attention to themselves and leading to a diagnosis of cystinuria. As a result, cystinuria is diagnosed more often in males than females.

Until recently it was presumed that males and females were equally likely to have cystinuria, although males more often get sick and are discovered. This situation seems to hold in Newfoundlands, where urine testing reveals an equal incidence of cystinuria in males and females despite the fact that more males than females are hospitalized with cystine uroliths. However, the situation is different in Mastiffs, where roughly 9 out of 10 dogs with positive urine tests for cystinuria are males.

As you can see, cystinuria is not a single disease. The sort of cystinuria that affects Newfoundlands differs in important ways from that which affects Mastiffs. Not surprisingly, cystinuria turns out to have a different genetic basis in these two breeds, too. Researchers recently identified the mutant gene responsible for cystinuria in Newfoundlands and found that the same mutant gene is not responsible for the disease in Mastiffs.

One other twist to the emerging story of cystinuria... It would seem intuitive that a dog with cystinuria would have cystine in his urine from birth, but that isn't always the case. Research studies have revealed at least a few dogs whose urine doesn't test positive for cystine until they're over a year old.

Cystinuria in Deerhounds

The first published report of cystinuria in a Deerhound that I'm aware of was a 1992 review in the *Journal of the American Veterinary Medical Association* that listed a Deerhound among 102 cases of dogs with cystine bladder stones between 1981 and 1989. No details were given.

Our Deerhound Health Survey in the mid-1990s revealed four cases of bladder stones in male Deerhounds, but the type of stone wasn't specified. In retrospect, some of these might have been cystine uroliths. In 1998, Richard Hawkins wrote me from Holland to describe two unrelated male Deerhounds (one an American import) that had been diagnosed with cystinuria. One hound had already undergone surgery to remove bladder stones. Then last year, Arline Schiff called to tell me about two more male Deerhounds—littermates this time—who had been diagnosed with cystinuria. Arline's encouragement prompted my first article on the subject. Since my article, several more affected Deerhounds have come to light—all males, and all of which required surgery to remove lodged cystine uroliths and save their lives.

The recent spate of Deerhounds with cystine uroliths has spawned a lively e-mail and telephone discussion among Deerhounds. Joan Creel took the initiative of contacting the University of Pennsylvania research team that had identified the cystinuria gene in Newfoundlands to see what help they might provide. Their response was positive and enthusiastic—specifically, they've agreed to work with the SDCA, through Joan as liaison, to embark on a cystinuria study in Deerhounds. What follows is a description of the study and how you can participate, kindly supplied by Joan.

The Deerhound Cystinuria Study

In the past year, the emerging picture of cystinuria in Deerhounds is sobering: We now know of several living deerhounds, all males, who have developed cystinuria and required surgery to save their lives. References to other affected deerhounds have been found in veterinary and research databases and literature. In addition, individuals within the Deerhound community have identified various affected Deerhounds they have owned or know of in the USA, Canada and Europe, some of which were mentioned in Dr. Dillberger's previous *Claymore* article. These dogs are from varied lines, suggesting that carriers now may be scattered across the Deerhound population.

While cystinuria is not among the "big three" health threats in our breed [bloat, osteosarcoma, and cardiomyopathy—JD], it is a concern worth addressing. We now have a unique opportunity to do just that.

OUR OPPORTUNITY

Researchers at the University of Pennsylvania School of Veterinary Medicine are seeking to identify the mutant genes and modes of inheritance behind cystinuria in different dog breeds. Dr. Urs Giger, Professor of Medicine and Chief of the Section of Medical Genetics of U Penn's Josephine Deubler Genetic Disease Testing Laboratory, heads this team. Dr. Paula Henthorn, PhD is his colleague in the Section of Medical Genetics and our primary contact with the research team.

This team and their colleagues have already identified the mutant gene for cystinuria in Newfoundlands and developed a blood test for carriers. They are now in the process of doing the same for Mastiffs, and are interested in doing the same for other breeds. They have determined that the mutant gene and mode of inheritance for cystinuria are not the same in all breeds, so it's important to study Deerhounds specifically.

This team, with Dr. Henthorn as our contact, has agreed to look for the mutant gene that causes cystinuria in Scottish Deerhounds.

The benefits to Deerhounds, breeders, and owners are significant. Once the mutant gene for cystinuria in Deerhounds is identified and a blood test for carriers is developed, we will have a tool to help guide breeding decisions and evaluate the potential risk of breeding pups who may inherit a mutant gene from both sire and dam. Used wisely, this tool could help minimize the numbers of future Deerhounds at risk for cystinuria.

HOW YOU CAN HELP

This research program is already funded. The only cost to us is \$18 for each Deerhound's initial urine test, which must be shipped for overnight delivery to the U Penn lab. The CYSTINURIA URINE SCREENING TEST SUBMISSION FORM and instructions for submitting this initial urine sample follow this article

Each sample will be analyzed using the Urine Nitroprusside Test for Cystine, and results will be mailed to you within a few weeks. Deerhounds that test positive are candidates for the ongoing study.

Valid research results depend on control of urine testing. That is why we are asked to submit urine samples for this research to the study group, so they can perform the same test on all samples.

[A quick test for cystinuria can be done in a veterinary office by centrifuging urine, sometimes after refrigeration and acidification, and examining the sediment for cystine crystals. Unfortunately, various factors can prevent crystals from forming in urine from a dog with cystinuria, giving a false sense of security. You should follow up a negative sediment test by sending a urine

sample to the U Penn lab for a confirmatory Urine Nitroprusside Test—JD.]

WHY YOU SHOULD PARTICIPATE

The first step in the study itself is to help the research team develop a sufficient data bank of urine samples from Deerhounds that tested positive, along with pedigrees and health histories for these dogs. Comparing pedigrees will give the team clues to what mode of inheritance may be involved in Deerhounds, while looking at health records may provide additional insights.

Once this has been accomplished with enough Deerhounds, the program can move to DNA testing of blood samples to find the mutant gene. When the gene is identified, a blood test for carriers can be developed.

WHAT ENROLLMENT INVOLVES

If you have a Deerhound that tests positive on the initial urine test, you will be asked to enroll it in the Deerhound Cystinuria Study and a confidential OWNER INFORMED CONSENT AND SAMPLE SUBMISSION FORM will be sent to you for completion and your signature. SAMPLE COLLECTION AND SHIPPING INSTRUCTIONS will accompany this form. The form and instructions are included after this article for your convenience.

At this point, you will also be asked to provide pedigree information and a health history in some detail. Later, follow-up urine samples and a blood sample to be stored for DNA analysis may be requested. All testing costs for enrolled Deerhounds will be paid for by the study, with no charge to owners.

PLEASE WORK THROUGH OUR SDCA REPRESENTATIVES

Dr. Dillberger is our professional contact with Dr. Henthorn. Joan Creel, who owns a young deerhound

who has tested positive but not yet developed stones, has volunteered to act as liaison between the Deerhound community and the research team. Working through Joan will help make interface logistics efficient and avoid burdening the researchers with questions. If you have questions or need help, please contact Joan at:

jcreel@neo.rr.com

Telephone: 330-836-7305

Telefax: 330-836-2582.

Breeding Recommendations

It seems likely that cystinuria is an autosomal recessive trait conveyed by a single mutant gene in Deerhounds. That means that:

- An affected dog should not be used for breeding because all his offspring will either have cystinuria or be carriers of the cystinuria gene.
- Parents of an affected dog are each a carrier of the cystinuria gene, and any offspring either produces will be at increased risk of being carriers, too. This fact should be weighed into decisions about using either parent for breeding again.
- Siblings of an affected dog are at increased risk of being carriers of the cystinuria gene, and that knowledge should be weighed into decisions about using them for breeding.

Before you use a hound for breeding you should submit a urine sample to U Penn for a Urine Nitroprusside Test and receive a negative result. Because some affected dogs test negative when young but positive when older, and because some adult dogs (at least Mastiffs) are intermittently positive as adults, a single negative test, especially in a young dog, is less than completely reliable. For that reason, I'd suggest that you test a dog before each breeding, even if s/he's tested negative before. Also, I would delay testing until adulthood, if possible.



The cystinuria study marks the SDCA's second research project into the mode of inheritance and genetic basis for a Deerhound disease. We began a similar investigation of osteosarcoma in Deerhounds several years ago by banking DNA samples from Deerhound families. While the osteosarcoma project may take a decade to bear fruit, the cystinuria study could yield results much faster. With the help of the University of Pennsylvania research team, Joan Creel, and the community of Deerhound owners and breeders, we have a good chance of discovering how cystinuria is inherited in our breed, finding the responsible gene, and developing a genetic screening test for the disease.

We deserve to be proud that a small breed club like ours has undertaken two major research projects aimed at giving us tools to breed better Deerhounds. In doing so, we can take our rightful place alongside past generations who have done their best with the tools at hand to be good breed stewards.

Dr. J. E. Dillberger, 5735 East Hidden Valley Drive, Reno, NV 89502-9676 (775) 857-3303

CYSTINURIA URINE SCREENING TEST SUBMISSION FORM

Use this form to submit an initial urine sample to be screened for cystinuria by the Nitroprusside Test.

Dog's Registered Name: _____ Breed: **Scottish Deerhound**

Call Name: _____ AKC#: _____ Chip #: _____

Date of Birth: _____ Sex (circle): M or F Reproductive status (circle): Intact or Neutered

Sire's Name: _____ AKC #: _____

Dam's Name: _____ AKC #: _____

Owner's Name (s): _____

Address: _____

Phone (day): _____ (evening) _____

Fax: _____ E-mail: _____

Date of Urine Collection: _____ Hours between dog's last meal and urine collection: _____

Current medications: _____

Diet being fed to dog: _____

Has your dog had previous urinary tract infections? Yes / No If yes, give details: _____

Sample Collection: Feed your Deerhound 2 to 4 hours prior to urine collection. Collect at least 3 to 5 milliliter (3-5 cc) urine in a very clean container—for example, a sterile glass or plastic tube with appropriate lids from your local veterinarian. If you are testing more than one dog, then you may store the liquid urine samples in the freezer until you have collected all samples for shipment.

Sample Shipment: Ship by overnight courier or US mail in an appropriate mailer, preferably with an ice pack. **Mail overnight only on a Monday, Tuesday or Wednesday.** Include this completed form and a check payable to Trustees, Univ. of Penn. for \$18 per dog. Mail to:

**Josephine Deubler Genetic Disease Testing Laboratory
Cystinuria Veterinary Hospital
Room 4020 University of Pennsylvania
3900 Delancey Street
Philadelphia, PA 19104-6010**

Phone: 215-898-8078, Fax: 215-573-2162, Secretary: 215-898-8880

Results: The results will be sent to you within 10 days of receipt of the samples. In case of an emergency, results can be faxed. **ALL identifying information is kept STRICTLY CONFIDENTIAL and is seen only by the University of Pennsylvania researcher team.**

"Deerhound Cystinuria Study"

URINE/BLOOD SAMPLE COLLECTION AND SHIPPING INSTRUCTIONS

Thank-you for participating in this study, which requires a DNA sample (in the form of blood) from your dog/patient. Participation in the study does not imply that a dog is affected with cystinuria nor that a dog is at risk of producing puppies affected with cystinuria. In order to investigate the genetic basis of cystinuria, it is necessary to obtain DNA and urine samples from unaffected relatives of affected dogs as well as from affected dogs themselves.

The urine and/or blood samples that you supply will be used to investigate the causes of cystinuria in dogs and to develop genetic approaches to detect carriers, which can help guide breeding decisions.

Blood Samples: Please provide whole blood in purple-topped (EDTA) blood tubes. For dogs weighing ≥ 5 lbs, draw 20 cc; for dogs < 5 lbs (2 kg), draw 10 cc/kg body weight. Rock tubes gently to distribute anticoagulant, but DO NOT centrifuge. If the blood sample will be shipped within 24 hours, then refrigerate until shipped.

Urine Samples: Please collect urine as a free catch while the dog is urinating. A new, clean disposable pie plate is a convenient collection vessel. Transfer urine to a standard urine sample container, available from a veterinarian's or physician's office. Please send between 3 and 10 cc of urine. (Please note that repeat urine specimens may be requested of dogs younger than 18 months of age.) Puppies may be stimulated to urinate by applying a wet cotton ball to the urogenital area.

Labeling Samples: Please label each sample with the dog's call name and the owner's last name.

Storing Samples: . If a blood or urine sample must be held for more than 24 hours before it's shipped, then please freeze the sample after placing it in a sealable plastic bag (in case the glass tubes break during freezing).

Include Appropriate Forms: Please include the following in your shipment:

- A signed and dated **Owner Informed Consent and Sample Submission Form**, for each dog from which a sample was taken,
- A **PEDIGREE** for each dog from which a sample was taken, and
- The sample(s) themselves.

Shipping: Place samples and forms in a small, insulated container, and include one or more frozen cold packs. Do not send samples on a Friday, as packages cannot be delivered on Saturday. Use the package label below to ship BY OVERNIGHT COURIER to:

**Dr. Paula Henthorn/Cystinuria
Section of Medical Genetics, 4006 VHUP
University of Pennsylvania
School of Veterinary Medicine
3900 Delancey St.
Philadelphia, PA 19104-6010
Phone no. (required by FedEx): 215-898-8894**

"Deerhound Cystinuria Study"

OWNER INFORMED CONSENT AND SAMPLE SUBMISSION FORM

Section of Medical Genetics, University of Pennsylvania School of Veterinary Medicine
Principal Investigators: Drs. Paula S. Henthorn and Urs Giger
Phone: 215-898-8894, FAX: 215-573-2162
<http://vet.upenn.edu/PennGen>

As the owner or his/her duly authorized agent, I wish to enroll (dog's name) _____ in a study to collect urine and DNA (from blood) from dogs affected with cystinuria and from their unaffected relatives, in order to discover the genetic basis of this disease and to develop genetic markers that could help eliminate the disease. My signature and date at the end of this document indicate that I have read the paragraphs below and understand the nature and terms of participation.

PURPOSE OF STUDY

I hereby grant permission for my pet to participate in a study designed to collect DNA (blood) and urine from dogs and their relatives affected with cystinuria, in order to determine the genetic basis for this disease.

DESCRIPTION OF PROCEDURES

I understand that in the course of this study, the investigators may obtain and use from my pet, blood, and/or urine to further their understanding of the disease and facilitate genetic testing. I consent to the use of the blood and urine samples, and will provide a pedigree and other requested information concerning my dog, provided that neither my animal nor I are identified in any publications, reports or presentations without my written authorization. I also give consent for the investigators to contact the individuals indicated below, who are the breeders of my dog or owners of dogs that are related to my dog.

RISKS ASSOCIATED WITH PROCEDURE

This study requires that 20 ml of blood (or 10 ml/kg body weight if my dog weighs less than 2 kg) be obtained from my pet to make DNA. The risk involved in drawing blood is minimal, but my dog may experience mild redness or bruising at the collection site. Additionally the hair may be clipped to facilitate visualization of the vein. I have chosen the veterinarian who will be performing this procedure, and will not hold the University of Pennsylvania responsible for any complications associated with drawing the blood.

TREATMENT AND POTENTIAL BENEFITS

I understand that there is no guarantee that my pet will benefit from its participation in this study. However, such participation may provide veterinarians with additional information and a better understanding of cystinuria and, ultimately, this may influence the course of treatment or genetic testing to help other animals in the future.

COSTS AND BENEFITS TO OWNER

In the event that DNA from my pet is used in the development of commercially available diagnostic markers or medical or surgical treatments, I understand and agree that any proceeds or benefits from such development are the sole and exclusive property of the University of Pennsylvania. I also understand that the University of Pennsylvania will not cover any charges that may be incurred for the drawing of blood.

CONFIDENTIALITY

I understand that any information about my pet, obtained from this study, will be kept confidential. No information by which my pet can be identified will be released or published without my written authorization.

AUTHORIZATION (Please circle to indicate your willingness to provide additional blood samples)

I am // I am not willing to provide additional blood samples if needed for research.

Dog's Registered Name: _____

Call Name: _____ AKC#: _____ Chip #: _____

Date of Birth: _____ Sex (circle): M / F Reproductive status (circle): Intact / Neutered

Breed: **Scottish Deerhound** Coat Color: _____

Sire's Name: _____ AKC #: _____

Dam's Name: _____ AKC #: _____

Owner's Name (s): _____

Address: _____

Phone (day): _____ (evening) _____

Fax: _____ E-mail: _____

Dog's Breeder's Name(s): _____

Address: _____

Phone (day): _____ (evening) _____

Fax: _____ E-mail: _____

I have read and understand the foregoing statements and agree to allow my pet to participate in this study. To the best of my knowledge, the information I have supplied above is accurate. Upon signing below, I am free to make a copy of this consent form.

Client/Owner/Agent's Signature: _____ **Today's Date:** _____
Client/Owner/Agent's Printed Name: _____

If submitting a urine sample, then please provide the following information:

Date of Urine Collection: _____ Hours between dog's last meal and urine collection: _____

Current medications: _____

Diet being fed to dog: _____

Has your dog had previous urinary tract infections? Yes / No If yes, give details: _____
