

# BULLDOG UPDATE

Volume 13 ■ Summer 2015

## Cystinuria Research in Bulldogs Focuses on Finding Gene Mutation

Veterinarian Nancy Newcomb of Elk City, Oklahoma, was surprised to learn the prevalence of cystinuria in Bulldogs. A potentially fatal disease that can cause painful cystine uroliths, or stones, leading to urinary blockage and possibly renal failure, cystinuria affects intact adult male Bulldogs.

The investigation of a promising marker test for Type III cystinuria, the type affecting Bulldogs as well as other breeds, by researchers at the University of Pennsylvania enabled Newcomb and other breeders to submit DNA samples, some taken from frozen semen. The results motivated the former member of the Bulldog Club of America (BCA) Health Committee to get involved in trying to help advance research to discover the Type III gene mutation.

In 2014, at the BCA National Specialty in Denver and at the combined Oklahoma City Bulldog Club and Texoma Bulldog Club of Wichita Falls, Texas, specialties, Newcomb and her team collected nearly 200 DNA samples. Meanwhile, the BCA Charitable Fund donated \$19,950 to the University of Pennsylvania to support the processing of the DNA samples. The research also has been supported by the

AKC Canine Health Foundation, the National Institutes of Health and other national parent clubs.

“It is now hard to find a male Bulldog that is negative for cystinuria,” Newcomb says. “Dogs suffer much like people with kidney stones, and they can go into renal failure if a urinary blockage is not relieved. It is highly possible that dogs die from undiagnosed cystinuria due to a lack of awareness and recognition of clinical signs.”

### A Highly Heterogeneous Disease

Cystine stones were first reported in dogs in 1823 during a necropsy discovery. Many years later in 1935, the first documented case of cystinuria was recorded in a dog with evidence of a metabolic defect related to elevated cystine levels in the urine and stone formation.<sup>1</sup> Researchers followed subsequent breedings of this dog and his Irish Terrier family and proved a genetic basis for cystinuria in dogs.

Cystinuria also occurs in humans. It was one of the first reported inborn errors of metabolism described in people more than 200 years ago. Inborn errors of metabolism are rare genetic disorders, usually caused by defects in specific proteins, or enzymes, that occur when the body cannot

properly metabolize food into energy. In people, two phenotypes have been identified. Mutations in the *SLC3A1* gene usually have an autosomal recessive mode of inheritance, while those in the *SLC7A9* gene often express a pattern of incomplete autosomal dominant inheritance. Mutations



have not been identified for about 10 percent of cystinuric human patients.<sup>2</sup>

Described as an inherited selective renal transport defect, cystinuria involves cystine, a relatively insoluble amino acid, and the dibasic amino acids ornithine,

*Continued on page 2*

New Expanded Classification System for Canine Cystinuria				
Phenotype	Type I-A	Type II-A	Type II-B	Type III
Inheritance	Autosomal recessive	Autosomal dominant	Autosomal dominant	Sex limited
Gene	<i>SLC3A1</i>	<i>SLC3A1</i>	<i>SLC7A9</i>	<i>SLC3A1</i> – Mastiffs (Bulldogs & French Bulldogs under investigation)
Sex	Males and Females	Males and Females	Males and Females	Intact Adult Males
Androgen dependence COLA	No	No	No	Yes
Breeds	Newfoundland Landseer Labrador Retriever	Australian Cattle Dog	Miniature Pinscher	Mastiff & Related Breeds Scottish Deerhound Irish Terrier Bulldog French Bulldog

## Cystinuria Research

From page 1

lysine and arginine, collectively known as COLA.<sup>3</sup> In normal dogs, about 99 percent of these amino acids are reabsorbed through the renal tubules. In dogs with cystinuria, the amino acids concentrate highly in the urine. Due to its low solubility in acidic urine, cystine predisposes dogs to forming cystine crystals and uroliths.

Researchers only recently realized the genetic heterogeneity, or diversity, of cystinuria in dogs. “We have proposed a new expanded classification system for canine cystinuria based on that in humans and due to multiple genetic etiologies and modes of inheritance,” says Paula Henthorn, PhD, professor of medical genetics at the University of Pennsylvania School of Veterinary Medicine. “Specific mutations within each type should lead to phenotypes that are similar so that the medical management and breeding advice applies to cases within that type.”

Henthorn and Urs Giger, DVM, PD, MS, DACVIM, DECVIM, DECVCP, professor of medical genetics, have studied cystinuria in dogs since the early 1990s. Their research has produced genetic tests for Type I-A, Type II-A and Type II-B cystinuria. Henthorn led development of the marker test for Type III cystinuria.

The classification system for canine cystinuria that Henthorn and her colleagues developed identifies four types:

- **Type I-A:** Has an autosomal recessive inheritance pattern, meaning an affected dog inherits a copy of the *SLC3A1* gene mutation from both the sire and dam. This severe type of cystinuria occurs in both males and females, though males are affected more frequently. A breed-specific direct DNA test in Newfoundlands, Landseers and Labrador Retrievers has helped reduce disease incidence as well as the number of carriers.
- **Type II-A:** Has an autosomal dominant inheritance pattern, meaning only one copy of the *SLC3A1* gene mutation is needed to cause the disease; however, the disease is more severe if the dog carries two copies of the mutation. Type II-A affects male and female Australian Cattle Dogs.
- **Type II-B:** Similar to Type II-A except the mutation occurs in the *SLC7A9* gene, Type II-B occurs in male and female Miniature Pinschers from a single pedigree of dogs from Europe.

- **Type III:** Although the gene mutation and mode of inheritance are not known, Type III occurs exclusively in intact adult male dogs. Besides Bulldog, this type affects French Bulldog, Mastiff, Scottish Deerhound, and Irish Terrier, and also may occur in additional breeds. Recent research has shown several DNA mutations together in the *SLC3A1* gene are associated with cystinuria in Mastiffs and related breeds, but not in Irish Terriers and Scottish Deerhounds. A DNA marker test is available in a few breeds to identify dogs at risk of stone formation, and one is expected soon for Bulldogs.

### Recognizing Clinical Signs

One study showed that the odds of a Bulldog developing cystine uroliths were 32 percent greater than for other breeds.<sup>4</sup> Many factors may influence the severity and variability of Type III cystinuria.

“Genetics and whether a dog is castrated likely contribute to a predisposition to develop cystine crystals and uroliths,” Henthorn says. “This type usually occurs later in life and is less severe, but this means carriers often have been bred and produced offspring before they are identified.”

A male dog’s anatomy plays a role in the development of urinary tract stones. “Males have a more narrow, less dilatable urethra than females,” explains Henthorn. “When urine passes through the os penis, the penis bone, stones can get lodged and cause an obstruction. Females are less likely to obstruct as their urethras are less narrow and more flexible.”

Type III cystinuria appears to be testosterone-dependent. “Only mature males are cystinuric, and castration completely resolves clinical signs of disease as urine amino acid concentrations revert to the normal range, as seen in females,” she says. “In breeds affected with this type, we recommend castration to reduce the formation of stones and the risk of blockage. This also will help reduce the frequency of the disease.”

Cystine stones usually form in the ureters, bladder or urethra. Urinary tract obstruction can be a life-threatening

## Does Your Bulldog Have Cystinuria?

The Metabolic Genetic Lab at the University of Pennsylvania performs a simple urine screening test to detect a risk for any type of cystinuria, though it does not detect symptomatic carriers of cystinuria-associated mutations.

[LEARN MORE](#)

The University of Pennsylvania also offers direct mutation-based tests for all Type I and Type II forms of cystinuria, as well as DNA marker test to identify male Mastiffs at risk for Type III cystinuria and early stone formation and female dogs that can transmit cystinuric-associated genes to their offspring. This test for Bulldogs and French Bulldogs is expected to be available later this year.

[LEARN MORE](#)

condition, thus owners of male Bulldogs are encouraged to frequently check to be sure they are urinating without problems. Signs, which can appear at any age at or after puberty, include straining or difficulty urinating and blood in the urine.

“In Bulldogs, it is so important to not breed affected dogs,” Newcomb advises. “You want to test your dogs and use the test results wisely. Carriers that test urine positive should not be bred, but carriers that are not positive can be bred to normal dogs. If no carriers were bred, there would be a risk of losing genetic heterogeneity in the breed.”

Henthorn agrees. “Our recent discoveries will have a major impact in the clinical management and genetic control of cystinuria in future generations. I am optimistic that we will learn more about cystinuria in the near future that will help Bulldog breeders manage this disease.” ■

<sup>1</sup> Henthorn PS, Giger U. Cystinuria. In: *The Dog and Its Genome*. Ostrander EA, Giger U, Lindblad-Toh K (eds). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press. 2006:349-364.

<sup>2</sup> Giger U, Brons AK, Fitzgerald CA, et al. Updates on Cystinuria and Fanconi Syndrome: Amino Acidurias in Dogs. *American College of Veterinary Internal Medicine Proceedings*. 2014.

<sup>3</sup> Brons AK, Henthorn PS, Raj K, et al. *SLC3A1* and *SLC7A9* Mutations in Autosomal Recessive or Dominant Canine Cystinuria: A New Classification System. *J Vet Intern Med*. 2013;27:1400-1408.

<sup>4</sup> Osborne CA, et al. *J Am Vet Med Assoc*. 2004 (June);204.

Purina appreciates the support of the Bulldog Club of America and particularly Elizabeth Milam, chair of the BCA Health Committee, in helping to identify topics for the *Purina Pro Club Bulldog Update* newsletter.

# Study Shows Proper Storage and Shipment of Semen Is Important for AI Breeding

**A**rtificial insemination (AI) gives breeders an opportunity to breed dogs that otherwise might not be possible due to geographical location, behavior incompatibilities or other factors. A recent study examined whether antibiotics added to commercial semen extenders to increase shelf life inhibit the growth of bacteria in semen samples.

“Breeders are not shipping females for natural breeding as much today and thus are turning more to AI,” says Ginny Altman, vice president of the American German Shepherd Dog Charitable Foundation. “Since semen collection introduces bacteria into the semen sample from normal bacterial flora, we wanted to learn whether the antibiotics used in extenders control the growth of bacteria.”

The study,<sup>1</sup> funded by the AKC Canine Health Foundation with support from the American German Shepherd Dog Charitable Foundation, was led by Carla Barstow, DVM, and Margaret Root Kustritz, DVM, PhD, DACT, professor of small animal reproduction at the University of Minnesota College of Veterinary Medicine. They recruited 14 male dogs from members of all-breed clubs in the Minneapolis area. Included were six Samoyeds, six Malamutes, one English Springer Spaniel, and one Labrador Retriever, ranging from 2 to 9 years of age.

“Our study was intended to mimic what can happen in the real world,” Barstow says. “It is important for the receiving veterinarian who will do the AI procedure and the brood bitch owner to know that the semen product they are using is safe. In reality, manufacturers include antibiotics in extenders to prolong shelf life, not inhibit bacterial growth.”

Commercial semen extenders are used with chilled and frozen semen. Extenders are liquid media that support spermatozoa by providing nutrients and a buffering capacity to offset changes in temperature that occur during storing and shipping.<sup>2</sup>

Chilled semen must be shipped and inseminated in a bitch within 24 hours of collection to retain viability and reduce the risk of disease. In addition to normal bacterial flora from a male dog’s urethra, semen can be infected from urine in the urethra and organisms that are shed from prostatic or testicular fluid caused by systemic infection.

Preventing disease transmission by AI will protect bitches only if an antibiotic is added to the semen. Two commercial canine extenders commonly used by theriogenologists, or reproduction specialists, were tested in the study. One extender contains several antibiotics, and the other has a single antibiotic.

Each semen sample was separated

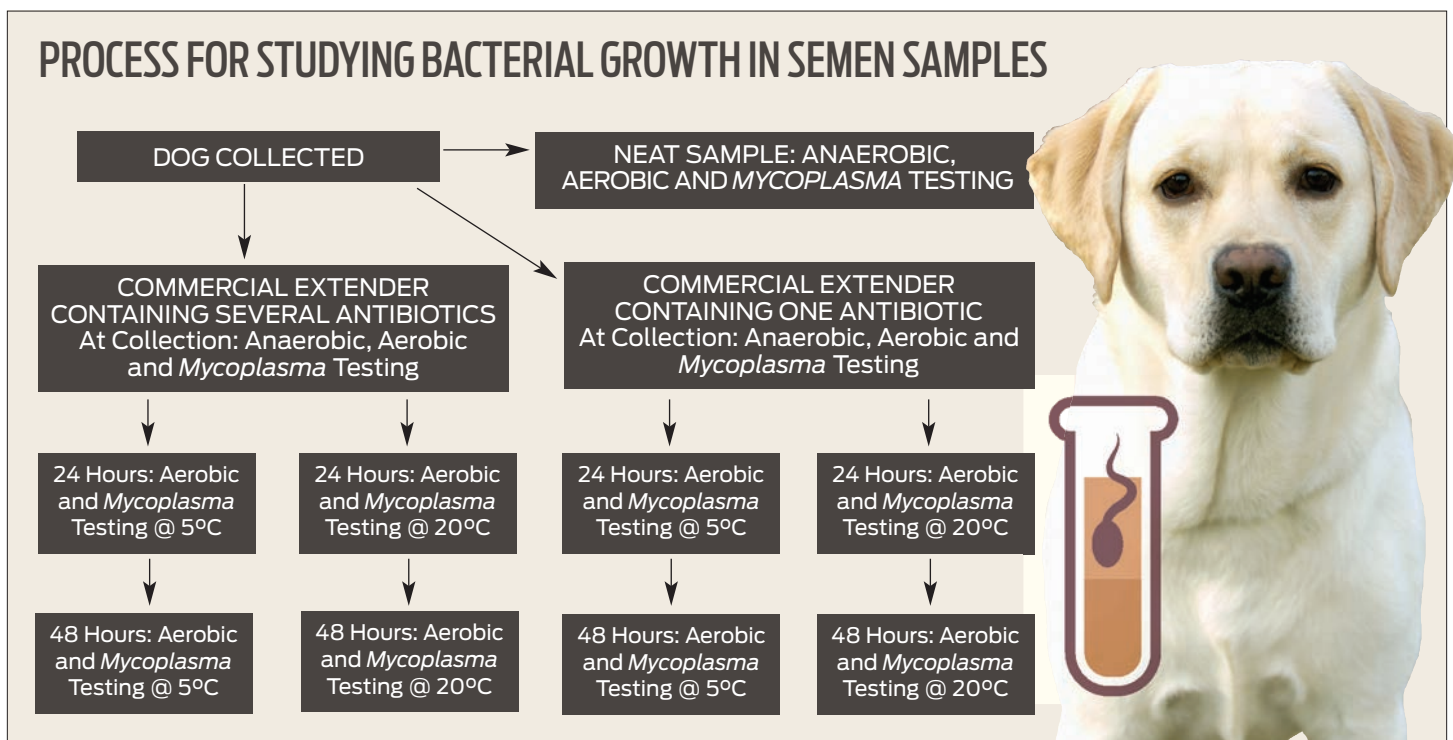
into 11 samples. Three cultures, considered neat samples, had no extender added and were tested for anaerobic, aerobic and *Mycoplasma* bacteria. The remaining semen was separated into two groups for adding the respective extender products. The individual extender groups were stored at room temperature (20 degrees Celsius) or refrigeration temperature (5 degrees Celsius) and tested for aerobic and *Mycoplasma* bacteria at collection, 24 hours and 48 hours.

Thirty-five percent of dogs had significant growth of bacteria in their semen, which is in accord with information from the literature, Barstow says. Bacterial growth was controlled in samples that were held at refrigeration temperature, but not in all the samples that were held at room temperature.

“Importantly, there was no significant growth in any refrigerated sample, which is the protocol for shipping chilled semen samples,” she says. “The question was whether extenders work as we thought they did, and yes, we showed they do.” ■

<sup>1</sup> Barstow C, Root Kustritz MV. Effects of Antibacterial Agents in Semen Extender on Bacterial Growth in Extended Canine Semen Held at 5°C or 20°C for Up to 48 Hours. *Clin Therio J.* 2014;6:231-237.

<sup>2</sup> Root Kustritz MV. *The Dog Breeder’s Guide to Successful Breeding and Health Management.* St. Louis: Saunders Elsevier. 2006.





## Upcoming Events

Check out upcoming Purina-sponsored show and sporting events at venues across the country. These events are great opportunities to meet dog enthusiasts, canine experts and Purina representatives who can answer questions about *Purina Pro Plan* dog food and *Purina Pro Club*.

[VIEW OUR CALENDAR](#)

## Follow the *Pro Plan* Champion Cup Leaders

View the Top 10 dogs leading the 2015 *Pro Plan* Champions Cup competition. The yearlong *Pro Plan* Champions Cup award is sponsored by *Purina Pro Plan* brand dog food and is based on points tabulated from Bests in Show and Group placements at more than 200 Purina-sponsored all-breed dog shows. The winner receives a \$10,000 cash prize, an original oil painting by dog portrait artist Linda Draper and a keepsake *Pro Plan* Champions Cup trophy.

[CHECK OUT THE STANDINGS](#)



## Purina Pro Plan Introduces NATURAL Dog Food

**P**urina Pro Plan dog food recently launched a new natural line. *Purina Pro Plan* NATURAL Formulas Plus Essential Vitamins and Minerals offer outstanding nutrition with

high-quality natural ingredients. This natural dog food contains no corn, wheat or soy; no added artificial colors, flavors or preservatives; and no poultry by-product meal.



*Purina Pro Plan* NATURAL includes three Adult Grain Free formulas: Chicken & Egg, Lamb & Egg, and Tuna & Egg. There also are two all life-stage formulas, Chicken & Brown Rice and Turkey & Barley, and one adult dog formula, Duck & Rice Formula.

[LEARN MORE](#)

## Want to Reach the Editor?

Have comments about the *Purina Pro Club Update*? Send them to us at: *Purina Pro Club Update*, c/o Editor, Nestlé Purina PetCare, 2T Checkerboard Square, St. Louis, MO 63164 or via email at [editor@purina.nestle.com](mailto:editor@purina.nestle.com).

## Looking to Reprint?

*Purina Pro Club Update* articles may be reprinted provided the article is used in its entirety and in a positive manner. To request permission to reprint an article, please contact the editor at: [editor@purina.nestle.com](mailto:editor@purina.nestle.com). Reprints should include the following attribution: Used with permission from the *Purina Pro Club Update* newsletter, Nestlé Purina PetCare Company.