PURINA PRO PLAN

BOXER Update

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BOXERS WITH DEGENERATIVE MYELOPATHY

MAY BENEFIT FROM DIAGNOSTIC BIOMARKER TEST

FIFTY-SEVEN PERCENT OF BOXERS ARE CONSIDERED AT RISK FOR DEGENERATIVE MYELOPATHY, RANKING THE BREED AMONG THOSE DEEMED HIGHLY SUSCEPTIBLE TO THE LATE-ONSET PROGRESSIVE NEURODEGENERATIVE DISEASE.

When their 9-year-old brindle Boxer "Lyric" (CH LattaLane's Irish Lyric) showed early-stage degenerative myelopathy (DM) in the spring of 2015, breeders Thomas J. and Carol Latta of Corder, Missouri, already recognized the signs. They had been screening their breeding Boxers for DM since the genetic test became available in 2009.

Not surprisingly, given the prevalence of DM in the breed, every Boxer in their breeding program had tested at risk for DM. However, Lyric was their first, and thus far only, Boxer affected by the disease.

Fifty-seven percent of Boxers are considered at risk for DM, ranking the breed among those deemed highly susceptible to the late-onset progressive neurodegenerative disease. First recognized in German Shepherd Dogs in the 1970s, the disease affects nearly 30 breeds plus mixed breeds. Adult dogs are usually around 9 years old when they develop DM.

A difficult disease to diagnose since it mimics other conditions, veterinarians rule out other diseases before determining if a dog has DM. A diagnosis is expensive because it

requires an MRI (magnetic resonance imaging) of the spinal cord. Even then, it is considered a presumptive diagnosis, as a definitive diagnosis is not possible until a dog dies and a necropsy and histopatholgy are performed.

Dragging or scuffing the hind legs is the first sign owners notice. Decreasing muscle control and weakness in the rear limbs lead to frequent falls and difficulty getting up. Usually within 11 months, a dog is paralyzed. The progressive disease spreads through the central nervous system, damaging the spinal cord, muscles, nerves, and brain.

"We began to notice that Lyric's rear paws occasionally knuckled over when she walked," says Thomas Latta. "A DM clinical trial was underway at the University of Missouri, so we contacted them to see if Lyric could take part because we live only an hour away and would be able to make the required checkup visits."

Lyric was accepted into a clinical trial designed to test a new therapy for the condition.

A PROGRESSIVE DISEASE

DM affects axons, as well as reduces the nerve insulation, or myelin in neuronal fibers. The process hinders signal transmission, resulting in hind limb clumsiness and loss of mobility. When the nervous system is no longer able to transmit secondary information or motor commands between the brain and hind limbs, a dog loses complete muscle function.

"The disease progresses to affect the front limbs, and then dogs go all the way down to where they are unable to walk and have difficulty swallowing as the brain stem becomes affected," says Joan R. Coates, DVM, DACVIM-Neurology, professor of neurology and neurosurgery at the University of Missouri. "It progresses from a disease that a neurologist would localize to the spinal cord to one that affects nerve and muscle function."

A longtime researcher of DM, Dr. Coates led the discovery in 2009 of the autosomal recessive gene mutation in the superoxide dismutase (*SOD1*) gene. This research, which was done in collaboration with the Broad Institute of MIT and Harvard and Gary Johnson, DVM, PhD, of the University of Missouri, found that the mutation responsible for DM in dogs is the same mutation that causes ALS (amyotrophic lateral sclerosis), or Lou Gehrig's disease, in humans.

ALS is named for the New York Yankee's Hall of Fame first baseman whose 16-year major league baseball career ended in 1939 due to ALS and who died from the disease in 1942. Knowing that DM and ALS are related led Dr. Coates and her team to begin investigating whether the diagnostics used in treating ALS patients also could be used for dogs with DM.

In Boxers, the DM allele frequency is 72 percent, meaning that the mutated gene is found on 72 percent of chromosomes carrying the alleles. To be affected, a Boxer must inherit a copy of the gene mutation from its sire and dam, though to be a carrier, only one copy of the mutation is needed.

Although the DNA test helps breeders make prudent breeding decisions, it has diagnostic limitations. Results from genetic testing allow breeders to breed dogs that carry the *SOD1* gene mutation to clear, healthy dogs to avoid producing affected dogs without reducing genetic diversity.

Dr. Coates also is the principal investigator of research leading to the recent discovery of a diagnostic biomarker that can be used to identify dogs at risk for developing DM. This research, published in the March-April 2017 issue of the *Journal of Veterinary Internal Medicine*, found that in dogs with DM, as in humans with ALS, a structural protein of motor axons contained in cerebrospinal fluid increases compared to older normal dogs and dogs with other mimicking spinal cord diseases.

This finding promises to help in the early diagnosis of DM. Meanwhile, two clinical trials at the University

SIGNS OF DEGENERATIVE MYELOPATHY

- Dragging or scuffing the hind legs
- Incoordination
- Decreasing muscle control
- Weakness in rear limbs leading to frequent falls
- Difficulty swallowing
- Paralysis

BOXER OWNERS CAN PARTICIPATE IN DM CLINICAL TRIALS

Owners of Boxers may help advance research of degenerative myelopathy (DM) by participating in clinical trials underway at the University of Missouri. The research team has two ongoing studies in which they are seeking Boxers 9 years of age and older when diagnosed. For additional information, you can visit the University of Missouri website or email lead investigator Dr. Joan Coates.

To qualify, a dog must be healthy and early in the disease process as confirmed by a board-certified veterinary neurologist. Owners must agree to return to the university for follow-up examinations.

Here is the criteria to participate:

- Slow, progressive loss of coordination over one to three months and not in discomfort
- Diagnostic testing showing no significant abnormalities on blood work, thoracic radiographs or abdominal ultrasound
- Normal spinal cord MRI (magnetic resonance imaging)
- Normal cerebrospinal fluid analysis
- Normal electrodiagnostic testing
- Genetic results showing homozygous for the *SOD1* gene mutation

of Missouri are evaluating effective therapies for dogs with DM. The one in which Lyric took part is a study to learn how injections of antisense oligonucleotides (ASO) slow the progression of disease. The other is a gene therapy study. Both therapies aim to suppress the SOD1 protein production. Here is a review of the recent progress to better understand DM.

THE pNF-H BIOMARKER

The ability to diagnose dogs with DM early in the disease process using the biomarker test will be helpful one day when there are effective therapies to help improve quality of life and life span. Working with Dr. Coates on the biomarker research were Michael Garcia, PhD, co-principal investigator and a graduate student in the Department of Biologic Sciences, and Christine Toedelbusch, DVM, a veterinary neurology resident and doctorate candidate. The research was funded by the AKC (American Kennel Club) Canine Health Foundation and the American Boxer Charitable Foundation.

In humans with ALS, an abundant structural protein found in the myelinated motor axons of nerve cells, called phosphorylated neurofilament heavy (pNF-H), was shown to have increased concentrations in blood and cerebrospinal fluid that aid in diagnosing the disease. The release of this protein occurs due to the degeneration of neural tissue and axons.

Based on this information, the research team began investigating whether this is true for dogs as well. "We hypothesized that pNF-H is readily detectable in the cerebrospinal fluid and blood of DM-affected dogs and thus could be a diagnostic biomarker for DM," explains Dr. Coates. "We found, however, that DM-affected dogs have higher levels of pNF-H in cerebrospinal fluid but not in their blood."

The study involved evaluating the cerebrospinal fluid and blood of DM-affected dogs, those with a confirmed diagnosis and those in the early stage of disease, as well as neurologically normal dogs, dogs with diseases that mimic DM, and asymptomatic dogs considered at risk for DM.

"Collecting cerebrospinal fluid is more complicated than collecting blood because it requires anesthesia, but it is less expensive than an MRI," Dr. Coates says.

The study showed that the biomarker test used to diagnose humans with ALS also can be used to diagnose dogs with DM. "As this test becomes more accessible to veterinarians and is used more frequently, it is possible we will begin to see reliable DM diagnoses from spinal fluid samples," says Dr. Coates.

EFFECTIVE THERAPIES

The two DM clinical trials underway at the University of Missouri have the same goal: to suppress the SOD1 protein production and thereby help treat affected dogs. One involves a molecular approach, and the other relies on gene therapy to help treat the disease.

"Part of the challenge in treating diseases of the central nervous system is getting past the blood-brain barrier, the protective barrier that separates the brain from the circulatory system, so the treatment can get into the spinal fluid and nervous tissue," Dr. Coates says.

The idea behind the molecular approach, or ASO therapy, is to disrupt the coding for SOD1 by using short strands of synthetic DNA as therapeutic agents. The theory is that ASO injections containing synthesized strands of nucleic acid will bind to the messenger RNA produced by the gene to silence it. A parallel study is underway in humans with SOD1-associated ALS.

"When ASO is injected into dogs or people, it suppresses the coding for SOD1 protein, thereby slowing or possibly halting the disease progression," explains Dr. Coates. "We want to define how long the ASO drug stays in the spinal fluid cells to help determine the frequency of injections."

The ASO study in dogs is funded by the National Institute of Neurological Disorders and Stroke, which is part of the National Institutes of Health, the AKC Canine Health Foundation, and the American Boxer Charitable Foundation.

Meanwhile, the gene therapy clinical trial involves using a small sequence of iRNA, or interference RNA, to suppress SOD1 protein production. This study, which is funded by the ALS Association TREAT ALS,™ involves giving dogs a one-time injection into their spinal fluid, with follow-up appointments in one month and then every three months.

MAKING STEADY PROGRESS

Lyric participated in the ASO clinical trial for 18 months. In December 2016, there was an unexpected change in her condition.

"We took Lyric to Dr. Coates and made the painful decision to say goodbye to our sweet girl," Thomas Latta says. "Although DM had affected her quality of life, in the end it was hemangiosarcoma that took her life."

Progress in treating and understanding DM is slow but steady. "The DNA test gives breeders a choice and aids their breeding program," says Dr. Coates. "These therapeutic treatments we are investigating are important, too, as is the ability to diagnose affected dogs early. DM is a heartbreaking disease, but one in which I am optimistic that we can have an impact in helping affected dogs and those who love them, and along the way help progress the clinical trial studies in ALS patients." ■

Purina thanks Dr. Joyce Campbell, chair of the American Boxer Club Health and Research Committee and a trustee of the American Boxer Charitable Foundation, for helping us to identify this topic for the *Boxer Update*.

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Have comments about the *Boxer Update*? Send them to Barbara Fawver, Editor, Nestlé Purina PetCare.

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