RESEARCH PROGRESS REPORT SUMMARY

Grant 02800: Defining the Effect of Genotype, Breed and Age on the Risk of Developing Canine Degenerative Myelopathy and Investigating the Molecular Mechanisms Underlying That Risk

Principal Investigator: Gary Johnson, DVM, PhD and Joan Coates, DVM, MS

Research Institution: University of Missouri

Grant Amount: $108,000

Start Date: 4/1/2020 **End Date:** 9/30/2021

Progress Report: End-Year 1

Report Due: 3/31/2021 **Report Received:** 3/1/2021

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Original Project Description:

Canine degenerative myelopathy (DM) is a progressive and inevitably fatal neurological disease affecting members of different dog breeds and mixes. It is an inherited disease with an age-related penetrance. The risk of developing the disease when dogs are homozygous for the causal SOD1 variant allele is currently unknown but of great concern to dog breeders and owners. The proposed research will further define the risk for developing DM in genetically at-risk dogs with a health survey distributed to dog owners whose dogs have been tested for the risk factor allele. This work will also examine the molecular mechanisms responsible for disease onset and spread by comparing single-nucleus RNA expression patterns in specific cell types in dorsal root ganglia from normal dogs and from affected dogs at various stages of the disease.

Publications:

Dr. Natalie Villani is a neurology resident and graduate student. The many of the experiments in Objective 1 are part of her thesis project. She is currently drafting a manuscript which we expect to submit for publication in the near future.

Presentations:

Dr. Joan Coates is scheduled to present a lecture about DM at the 2021 AKC Canine Health Foundation National Parent Club Canine Health Conference. She intends to include much of the information from this projects at that time.

Report to Grant Sponsor from Investigator:

This project has two objectives. The first objective is to generate and interpret empirical evidence about the likelihood that dogs will develop DM, if and when they reach old age. To do this, we sent invitations to the owners of nearly 10,000 dogs to provide us with follow up clinical information about their dogs via an online survey. To be considered for participation in this survey, the dogs had to meet two criteria. First, the dogs should have been DNA-tested for DM before they were 5 years old. When tested, these dogs were too young to show signs of DM. Thus, their owners' decision to test could not have been biased by prior knowledge of the dogs DM status. Second, the dogs should have been 8 years old or older when surveyed. Thus, the dogs would be old enough to exhibit the clinical signs of early-onset DM.

We received an approximately 25% response to our survey request. After review, the clinical records for 2,143 dogs were eligible for the final analysis. We found that dogs that with homozygous "at risk" DM-test result, began to develop a DM-like disease by 7 years of age. By 12 years of age, over 50% of them developed a DM-like disease and over 60% of them developed a DM-like disease as they got older. This confirmed an earlier conclusion reported in 2014 from a similar study with a cohort of 512 dogs. For the first time, we showed empirical evidence that heterozygous dogs with the "carrier" DM test result had an increased risk of developing DM starting before they reached 12 years of age. Nonetheless, fewer than 5% of these dogs developed a DM-like disease by 14 years of age. Thus, we have not modified our conclusion that, in general, offspring resulting from matings involving at least one homozygous SOD1:c.118G allele dog are very unlikely to develop DM. Nonetheless, the risk "carrier" dogs developing DM was not the same in all breeds and we may need to modify our recommendations to the breeders of certain breeds at higher risk.

We found that the risks for developing DM in female dogs was almost identical to the risks for developing DM in male dogs.

For the first time, we showed empirical evidence that a dog's breed influences the age at onset of DM. There were sufficient numbers of survey records to statistically compare ages at onset of DM for six dog breeds: Bernese Mountain Dog, Boxer, Chesapeake Bay Retriever, German Shepherd Dog, Pembroke Welsh Corgi and Rhodesian Ridgeback. We found that the Rhodesian Ridgebacks and the Bernese Mountain Dogs had the earliest onsets, while Pembroke Welsh Corgis had the latest onsets.

The second objective was to study stored tissue, collected from owner-requested euthanasias of DM-affected and DM-free dogs, with the goal of learning about molecular mechanisms underlying start and

progression of DM. We focused on dorsal root ganglia since these anatomical structures contain the neurons that provide the brain with sensory information necessary for normal gait and abnormal hind-limb gait is the first clinical sign of DM. We are using a technology known as single-nucleus RNA sequence analysis. This technology provides an indication of the patterns of genes that are turned on in the individual nuclei from cells in a tissue sample. This is our first experience with this technology and progress has been slow due to technical problems and laboratory shut downs because of the COVID-19 pandemic. Nonetheless, our latest efforts show promise and we expect progress to accelerate as we continue the study. Our hope is that our experiments will lead to a better understanding of DM-disease mechanisms, thereby identifying druggable targets leading to the development of therapies that delay the onset or slow the development of DM.