



RESEARCH PROGRESS REPORT SUMMARY

Grant 02458-A: A Laboratory Test for Detecting Drug Resistance in Canine Heartworm Disease

Principal Investigator: Matt Brewer, DVM, PhD
Research Institution: Iowa State University
Grant Amount: \$15,000
Start Date: 2/1/2018 **End Date:** 1/31/2020
Progress Report: FINAL
Report Due: 1/31/2020 **Report Received:** 3/28/2020

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Original Project Description:

Dirofilaria immitis is the nematode parasite that causes heartworm disease in the United States. Heartworm infection causes severe pathology and suffering in dogs and cats. Until recently, heartworm infection was a preventable disease due to the availability of effective monthly preventative treatments. A recent development shows drug-resistant heartworms have emerged in the United States. The scope of the resistance issue has not yet been characterized because there is a critical need to develop a test that can discriminate drug-susceptible and drug-resistant parasites. Recent research assessed computer-aided motility studies of the parasite in the presence of drugs, however, there are no motility differences among parasite isolates in these assays. The investigators have developed biochemical stains and measurements that can quantify parasite killing in the presence of anti-parasitic drugs. In this study, the investigators will evaluate various metabolic assays and staining procedures to compare drug-susceptible and drug-resistant heartworm isolates in an effort to identify the best assay for detecting heartworm killing, and thereby creating a tool to rapidly identify resistant infections in dogs.

Publications:

Chelladurai, Martin, Vargas, Castro, Kaplan, Brewer. Laboratory assays reveal diverse phenotypes among microfilariae of *Dirofilaria immitis* isolates with known macrocyclic lactone susceptibility status. PLOS Neglected Tropical Diseases. (Under review)



Presentations:

Jesudoss JR, Brewer MT. Biochemical testing reveals diverse phenotypes of *Dirofilaria immitis* microfilariae. ISU-CVM Vector Biology Scientific Working Group. December 2019.

Jesudoss JR, Magiera JM, Martin KA, Brewer MT. Evaluation of biochemical assays for the in vitro testing of drug responses in the canine heartworm *Dirofilaria immitis* microfilaria. American Society of Parasitologists, Rochester, MN. July 2019.

Jesudoss JR, Magiera JM, Martin KA, Brewer MT. Evaluation of biochemical assays for the in vitro testing of drug responses in the canine heartworm *Dirofilaria immitis* microfilaria. World Association for the Advancement of Veterinary Parasitology, Madison, WI. July 2019.

Jesudoss JR, Magiera KM, Martin KA, Brewer MT. Evaluating laboratory tests for detecting drug resistance in the canine heartworm *Dirofilaria immitis*. Iowa State University Research Symposium, Ames, IA. March 2019.

Magiera KM, Jesudoss JR, Martin KA, Brewer MT. Evaluating laboratory tests for detecting drug resistance in the canine heartworm *Dirofilaria immitis*. ISU-CVM Research Symposium, Ames, IA. August 2018.

Report to Grant Sponsor from Investigator:

Drug resistance has been confirmed in the heartworm *Dirofilaria immitis* and this is a major challenge for canine health. This means that there are isolates of heartworm circulating in the United States that are capable of infecting dogs even if they have been receiving heartworm preventative medication.

One problem for veterinarians is that it is not possible to determine if a dog's heartworm infection is drug –susceptible or –resistant with a single blood sample. Our goal is to move toward developing such a method.

Our hypothesis is that larval parasites (microfilariae) found circulating in peripheral blood will respond differently when exposed to antiparasitic drugs in the laboratory. In this pilot study, we evaluated 7 different types of laboratory techniques to evaluate parasites. These assessments measured a variety of factors such as cell membrane leakage, metabolism, and cellular proteins that may pump drugs out of the organism.

The key findings of this study are that we could detect differences among drug-susceptible and –resistant parasites in all 3 categories of lab assessments. Because of this exciting progress, we began to expand the number of strains tested beyond those in the original proposal. These experiments



revealed that not ALL resistant strains behaved exactly the same in our assays. This is not surprising, since there are likely multiple mechanisms for drug resistance.

Our results are an exciting first step, and they are currently under review for publication in an open access scientific journal so that our data will be widely available for other scientists. In the current project, we used a fairly narrow range of incubation conditions (time, temperature, humidity) and the main variable tested was quantity of antiparasitic drug. Going forward, the next step is to further optimize assays showing the most promise by varying incubation conditions and expanding the number of strains tested. We also need to test the effect of the time between blood collection and evaluation; in this study samples were always assessed within 24 hours and this is not always possible under field conditions.

In summary, this research project involved veterinary student researchers and produced significant results. We appreciate the investment that AKC CHF has made in this project and we hope to continue to partner with the foundation as we pursue this important work.