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Abstract

Multiple anthelmintic drug resistance (MADR) has been confirmed in canine hookworms, *Ancylostoma caninum*, from Greyhounds across the US as well as a small number of non-Greyhound pet dogs in Florida and Georgia. However, we do not know at the present time how prevalent MADR is in hookworms from the latter population, or even the prevalence of single anthelmintic drug resistance in this group. *A. caninum* are blood-sucking intestinal nematodes that can cause anemia in young puppies and heavily infected adult dogs, potentially resulting in death. The larval stage can also infect people, causing itchy tracks in the skin (cutaneous larva migrans). For the protection of animal and public health, it is essential that veterinarians ensure *A. caninum* infections are successfully eliminated following anthelmintic treatment to avoid further selection for resistance and subsequent transmission of resistant hookworms to other dogs. By determining just how common single- or multiple-resistant hookworms are in the general dog population, we can inform veterinarians of the likelihood of treatment failure in their patients and the heightened zoonotic risk, which will prompt closer monitoring of post-treatment fecal egg shedding and thus prevent continued spread.

This project aims to establish the frequency of single nucleotide polymorphisms (SNPs) in the beta-tubulin-1 gene of hookworms infecting north central Florida non-Greyhound dogs. Three such SNPs have been shown to be associated with benzimidazole resistance: a SNP in codon 167 for *A. caninum,* and SNPs in codons 198 and 200 in other veterinary nematodes. Feces will be collected from pet dogs and shelter dogs to screen for *A. caninum* infection. Infected dogs (at least 10 pet dogs and 10 shelter dogs) will be treated with a standard course of the benzimidazole drug, fenbendazole (50 mg/kg PO once daily for 3 days). Feces will be collected 14 days post-treatment and examined for hookworm eggs. Egg counts will be performed on both pre- and post-treatment feces to calculate the fecal egg count reduction, with a value of < 75% being consistent with resistance. SNP frequencies for codons 167, 198, and 200 will be determined for each sample at each time point by performing quantitative PCR (qPCR) using published protocols (Schwenkenbecher et al. 2007, Schwenkenbecher and Kaplan 2009). In addition, frozen eggs previously collected from 31 shelter dogs treated with the anthelmintic pyrantel (from the pyrimidine drug class) will be analyzed to determine whether dogs resistant to pyrantel are more likely to have higher frequencies of the benzimidazole resistance-associated SNPs, suggesting MADR.

The specific aims of this project are:

1) Quantitate the fecal egg count reduction in non-Greyhound dogs in north central Florida treated with fenbendazole to determine whether their hookworms demonstrate resistance.

2) Using qPCR, establish the frequency of SNPs in codons 167, 198, and 200 in hookworms that show resistance versus susceptibility to fenbendazole.

3) Determine if *A. caninum* that are resistant to pyrantel are also more likely to possess benzimidazole resistance-associated SNPs.

The student on this project will be trained to carry out fecal flotation exams, parasite identification, fecal egg count reduction testing, DNA extraction, and quantitative PCR. They will be responsible for sample collections and all testing procedures, with assistance provided by a Biological Scientist as needed. They will also participate in data analysis, and draft a poster and research manuscript under the mentorship of the PI. To ensure samples are available for testing during the FVSP project period, sample collection from pet and shelter dogs will begin in the spring and continue into the summer. The egg