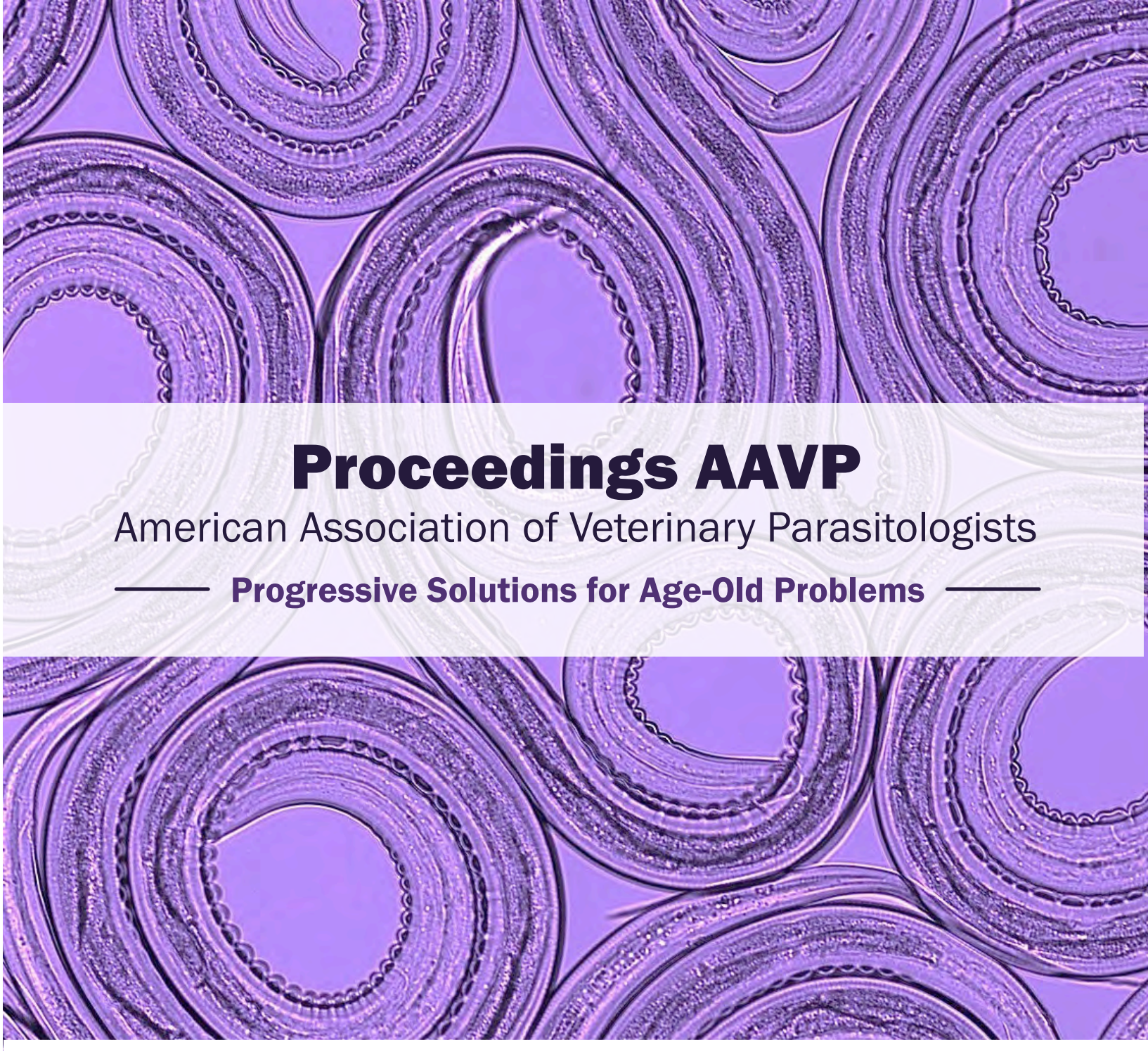

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Parasitic nematode beta-tubulin alleles cause benzimidazole resistance and affect organismal fitness

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Parasitic nematodes are major burdens on livestock production around the world. Anthelmintic drugs are the first tool to fight these infections, and resistance to these drugs continues to increase worldwide. To fight this resistance, we must thoroughly understand the genetics and mechanisms of resistance. The commonly used benzimidazoles (BZ) represent the most well understood, however, we still do not know the mechanisms of resistance and all of the genes involved. Three well known parasite beta-tubulin alleles (F200Y, E198A, F167Y) in a nematode-specific beta-tubulin gene have long been identified in resistant parasite populations. Recent advancements in sequencing technologies have allowed the identification of a number of new parasite beta-tubulin alleles, two of which we have included in our study, E198V and E198L. We independently introduced all five of these alleles into the *ben-1* gene of the BZ-susceptible free-living nematode Caenorhabditis elegans. The genome-edited strains were exposed to either albendazole (ABZ) or fenbendazole (FBZ) in high-throughput assays that measure nematode responses to the BZ compounds. We performed these assays across a range of drug concentrations to quantitatively measure BZ resistance. All five alleles convey a similar level of ABZ and FBZ resistance as found in a deletion of the entire *ben-1* gene. Another essential aspect of resistance control, is understanding the long term fitness effects of these alleles. We found that the E198V allele was resistant to BZs but was less fit in control conditions. Our results validate that the identified alleles in parasite beta-tubulin genes confer resistance. Additionally, we found that rare alleles in parasite species confer fitness consequences in comparison to other resistance alleles.

Small Ruminant Nematodes

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Carvone modulates *in vitro* and *in vivo* the kinetic behaviour and efficacy of abamectin

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The search of novel strategies to control gastrointestinal nematodes in ruminants is a concern considering the increasing of anthelmintic resistance. Bioactive phytochemicals may contribute to improve parasite control by enhancing the effect of existing anthelmintic drugs. This work assessed the *in-vitro* and *in-vivo* pharmacological interaction and the *in-vivo* efficacy of abamectin (ABM) combined with the plant-derived compounds carvone (CNE), in lambs naturally infected with resistant gastrointestinal nematodes. At first, the modulation of P-glycoprotein (P-gp) by CNE was assessed using the intestinal explant model. Rhodamine-123 (Rho123) and ABM were used as substrates to measure their accumulation in cattle ileum in

presence or absence of CNE. For the *in vivo* assay, twenty-eight (28) lambs were allocated into three (3) experimental groups. Each group was treated orally with either ABM (0.2 mg/kg), ABM in combination with CNE (100 mg/kg, four doses every 24 h) or remained as untreated control. Blood samples were collected between 0 and 168 h post-treatment and plasma levels of both compounds were determined by HPLC. Individual fecal samples were collected on days -1 and 14 post-treatment to perform the fecal eggs count reduction test. The presence of CNE increased significantly ($P<0.05$) Rho123 and ABM accumulation in the intestinal explants. CNE coadministration prolonged ABM absorption in lambs. ABM T½ ab. were 1.57-fold longer ($P<0.05$) in the co-administered group. Concentrations of CNE between 420 and 2593 ng/mL were detected in the bloodstream between 1 and 48 h post-treatment. The *in-vivo* efficacy of ABM against gastrointestinal nematodes increased from 94.9% to 99.8 in the presence of CNE. In-vitro / i*n-vivo* pharmaco-parasitological studies are relevant to corroborate the interactions and the efficacy of bioactive natural products combined with synthetic anthelmintics.

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Developing the nemabiome as an alternative to fecal egg counting: Absolute quantitation of parasitic nematode DNA in fecal samples

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Routine helminth diagnostics in parasitology laboratories largely relies on microscopy methods that are labor-intensive, lack sensitivity and are difficult to standardize. We recently developed "Nemabiome" sequencing which determines the relative quantitation of parasite species using eggs or larvae harvested from fecal samples. We are now developing absolute DNA quantitation of parasite species in fecal stool samples using the sheep GI nematodes as a model to develop this approach and provide proof of concept for other host-parasite systems. The basic approach is to apply ITS-2 rDNA nemabiome sequencing to fecal samples spiked, prior to genomic DNA extraction, with accurate quantities of synthetic DNA comprising 500 bp of random sequence with terminal sequence tags complementary to primers used for parasite ITS-2 rDNA amplification. Parasite-derived Illumina read counts are then normalized to the synthetic DNA internal standard-derived DNA counts. The first step will be to use purified *Haemonchus contortus* eggs to establish a spike-in concentration that provides an appropriate ratio of *H. contortus* ITS-2 rDNA reads: spike-in synthetic DNA reads. We will then undertake experiments to determine the quantitative relationship of the normalized *H. contortus* nemabiome read count with egg numbers. Once optimized, the method will then be applied to genomic DNA directly prepared from fecal samples from sheep experimentally infected with *H. contortus* to test and validate its use directly on "stool DNA" samples. Finally, it will be tested on fecal samples from sheep in the field that contain mixed species infections with known fecal egg counts to further validate the technique and relate "DNA content" values to the more commonly used fecal egg count data. If, successful, this assay will allow quantitation of parasite infection intensities using molecular biology work flows without the need for microscopy-based fecal egg counting.