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ABSTRACT BOOK

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Viasoft Experience

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and results were compared with more accurate PacBio SMRT and Illumina amplicon sequencing data. The validated assay was then applied to a large geographically diverse set of *Haemonchus contortus* and *Ancylostoma caninum* populations. F167Y and F200Y SNP frequencies were very consistent across replicates for the *N. battus* validation and comparable to the Illumina and PacBio sequencing results. Application of the validated ONT amplicon sequencing to the full-length isotype-1 β-tubulin gene from *Haemonchus contortus* and *A. caninum* populations detected additional non-synonymous SNPs which will be presented. ONT long-read amplicon sequencing is reliable for low frequency SNP detection in parasite populations and uncovers additional non-synonymous isotype-1 β-tubulin SNPs.

Development and field-evaluation of a deep amplicon sequencing assay to detect the levamisole-resistance associated SNP (S168T) in the *acr-8* gene

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A non-synonymous Single Nucleotide Polymorphism (SNP) in (S168T) in the *H. contortus acr-8* gene has been shown to be associated with levamisole resistance. The aim of this work was to develop a deep amplicon sequencing assay to detect this SNP in ovine GIN field populations, and to determine its frequency in archived samples whose levamisole resistance phenotype had previously been established using a Larval Development Assay (LDA). Primers designed to amplify the relevant *acr-8* fragment from three main ovine GIN species, *H. contortus*, *T. circumcincta* and *T. colubriformis*, were tested against single species isolates to determine specificity. Illumina Miseq paired-end sequencing was performed on amplicons from various field populations and a bespoke DADA2 based denoising pipeline developed to generate Amplicon Sequence Variants (ASVs). ASVs were taxonomically assigned to GIN species reference sequences, and the S168T SNP frequency determined for each species. The sequencing assay was used to determine the S168T SNP frequency in US field populations of known levamisole resistance phenotype. A total of 68 USA farm samples submitted to the University of Georgia (2015 and 2019) were classified as either susceptible (n=18), suspected-resistance (n=7), low-resistance (n=27) and high-resistance (n=16) by LDA. The mean frequencies of the S168T SNP, combined for each GIN species, were 10.9% and 32.9% in the susceptible and high-resistance groups, respectively. We suggest that the detection of the S168T SNP in "susceptible" populations and the moderate overall correlation between the LDA resistance phenotype and S168T SNP frequency ($R^2 = 0.4288$) likely results from limitations in LDA sensitivity. Finally, field populations from UK, US, Canada and Greece were used for further validation and the presence and frequencies of the S168T SNP broadly reflected the levamisole usage histories in the different geographical regions.

Unraveling benzimidazole resistance in cattle gastrointestinal nematodes through Next-Generation Sequencing

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Gastrointestinal nematodes (GIN) infection represents one of the most significant health challenges in ruminants and are the primary cause of economic losses in livestock production systems worldwide. Their control relies almost exclusively on the use of synthetic antiparasitic compounds. Inappropriate use has led to therapeutic failures associated with the development of resistant nematode parasites. The advancement in high-throughput sequencing technologies has enabled the development of molecular-based techniques for resistance diagnosis. This study describes the first molecular identification of GIN parasitizing cattle across 6 commercial farms located in Argentina, using the ITS-2 gene metabarcoding. Additionally, the fecal egg count reduction test and sequencing of the β-tubulin isotype-1 gene were used to assess benzimidazole (BZD) resistance under different anthelmintic treatment regimens (BZD alone or BZD+macrocyclic lactones). Seven GIN species were identified: *H. placei (64.1%), C. punctata (26.6%), O. radiatum (3.6%) O. ostertagi (3.5%)*, *H. contortus (1.1%), C. oncophora (0.9%) and T. axei (0.2%).* Among the 21 anthelmintic treatments applied across six farms, two farms exhibited overall efficacies above 95% for all treatments, while four farms displayed efficacies below 95% for either BZD alone or combined treatments. While *Cooperia punctata* and *Ostertagia ostertagi* were the main species resistant to BZD, *Haemonchus placei* was found to be BZD-susceptible on all the farms. BZD resistance associated SNPs in codons 167, 198 and 200 of the isotype-1 β-tubulin gene were present in both *C. puntacta* and *O. ostertagi*, being the F200Y allele recovered with the highest frecuency. Finally, BZD resistance associated SNPs were found at low frequencies even when the *in vivo* FECR was >95% on the field, demonstrating the potential of β-tubulin amplicon sequencing to screen for the early emergence of resistance mutations. Monitoring the prevalence and distribution of tubulin gene polymorphisms is crucial for tracking the emergence and spread of BZD resistance, which is now being used for the first time (as a model) in the large extension cattle ranches of the *Argentina´s Pampa Húmeda*.

Nemabiome metabarcoding shows variable resistance levels and species dynamics in anthelmintic resistant gastrointestinal nematode populations

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Gastrointestinal nematodes (GINs) pose a major threat to livestock production by reducing yields in milk, meat, and wool. Although faecal egg count reduction tests (FECRTs) measure phenotypic resistance to anthelmintics, they do not reveal the underlying dynamics and diversity of the resistant GIN populations. This study aimed to elucidate the species composition and diversity of anthelmintic-resistant GIN populations in sheep farms in southeast England by integrating molecular techniques with standard FECRTs. Eighteen farms were sampled, with faecal collections from three groups of 10 lam-