Title: Rapid Molecular Phenotyping of Triclabendazole Resistance in *Fasciola hepatica* Using LAP-MALDI Mass Spectrometry

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ABSTRACT

Fascioliasis, caused by the fluke *Fasciola hepatica*, remains a major constraint on livestock production and an emerging human health concern. Control relies heavily on the anthelmintic drug triclabendazole (TCBZ). However, widespread resistance to TCBZ threatens the long-term effectiveness of current treatment strategies. While genomic and transcriptomic studies have provided valuable insights, there remains a critical need for protein-level approaches capable of resolving resistant *F. hepatica* phenotypes in biologically and field-relevant samples. We have previously utilized in-depth GeLC proteomic profiling to characterize molecular differences between triclabendazole-susceptible (TCBZ-S) and triclabendazole-resistant (TCBZ-R) *F. hepatica* isolates, establishing resistance-associated variation at the proteome level. Building on this framework, Liquid atmospheric pressure MALDI mass spectrometry (LAP-MALDI-MS) was applied as a rapid, chromatography-free platform for molecular phenotyping and biotyping of isolates. Liver fluke samples representing somatic, extracellular vesicle (EV), and EV-depleted protein fractions were analyzed from four isolates with contrasting susceptibility profiles: two TCBZ-S (Aberystwyth and Italian) and two TCBZ-R (Penrith and Kilmarnock). LAP-MALDI generated highly reproducible lipid- and proteoform-rich spectral fingerprints across all fractions. Although no single diagnostic resistance marker was evident by visual inspection, multivariate statistical analysis (PCA/LDA) consistently resolved TCBZ-S and TCBZ-R isolates across somatic and secretome (EV and EV-depleted) fractions, indicating that resistance-associated information is encoded as distributed multivariate signatures. Inclusion of the rumen fluke *Calicophoron daubneyi* as an outgroup confirmed robust species-level discrimination. Targeted top-down proteomics of multiply charged ions enabled direct identification of biologically relevant proteins, including helminth defence molecule-1 and thioredoxin. Together, these data establish LAP-MALDI-MS as a rapid phenotyping platform that complements classic bottom-up proteomics, with strong potential for translational diagnostics and resistance surveillance.