Sustainable worm control strategies for gastrointestinal nematodes and liver fluke in ruminant livestock

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Helminth parasites include many of the most important pathogens infecting humans and animals worldwide. These parasites reduce the food conversion efficiency of their livestock hosts, either by evoking immune responses, or directly, resulting in production loss through reduced weight gains and deaths. Effective control strategies are therefore a global priority.

The principles of control of GI nematodes and liver flukes are well established, with the aim of reducing host exposure to infective third stage larval (L_3) or metacercarial stages to a level that will allow for the development of protective immunity, while enabling parasitic stage burdens to be manageable. This involves integrated evasive host management and the use of anthelmintic drugs to interrupt the parasites' life histories and thereby suppress pasture contamination with infective stages. In the control of *Fasciola*, anthelmintics are used to prevent the shedding of those particular eggs that would otherwise give rise to metacercarial challenge; the timing of drug treatments being determined by the climatic and environmental drivers of the annual life cycle of the parasites. In the control of GI nematodes with direct life cycles and multiple annual generations, drugs are given to reduce the shedding of eggs to a level that will give rise to L_3 challenge that is sufficient to induce slow onset and long lasting protective immunity, while not reaching a level at which consequential production loss occurs. The timing of these treatments is governed by the annual production cycle of the host, giving rise to the seasonal presence of naïve or less immunologically capable animals; and climatic conditions favouring larval development and L_3 survival. These principles are generally poorly understood, or considered to be too complex to be practical, hence most livestock keepers administer anthelmintic drugs with the simple goal of removing all parasites from their animals. This strategy has historically been effective in preventing clinical disease and deaths, but often fails where the objective is to optimise production efficiency as is required to meet global needs for food production in accordance with UN Sustainable Development Goals

Helminth parasites are highly complex organisms with large genomes, high levels of genetic polymorphism, and enormous biotic potential. It is therefore inevitable that they will evolve in response to changing climatic conditions that are favourable for environmental stages, and exposure to anthelmintic drugs affecting parasitic stages. There are many examples of changing geographical and temporal distributions of GI nematode and fluke parasites, of GI nematode resistance to each of the four single active broad spectrum anthelmintic drugs, and of *Fasciola* resistance to triclabendazole. Benzimidazole resistance in *Teladorsagia circumcincta* has reached a state of genetic fixation in some sheep flocks, whereby the drug can no longer be used.

Current strategies to reduce the emergence and spread of anthelmintic resistance in GI nematodes involve: ensuring that nematodes are exposed to an effective anthelmintic drug concentration; consideration of the timing and frequency of treatments so that only a small proportion of the total effective parasite population is exposed to the anthelmintic; and treatment of introduced animals with effective anthelmintic drugs, or combinations, to limit the introduction of resistant individuals into otherwise susceptible populations. Refugia based strategies are based on mathematical modelling of resistance allele frequencies in parasite populations. However, aspects of gastrointestinal nematode biology and the genetics of resistance mutations that are important in these models are unknown. Sensitive molecular markers and platforms for their application are needed to monitor the effects of these practices on the frequency of resistance alleles within nematode populations; enabling definition and refinement of sustainable gastrointestinal nematode control. In the meantime, the focus of GI nematode control has shifted towards the integration of evasive management and strategic or targeted anthelmintic drug treatments, aimed at maintaining adequate productivity in the face of a low level of larval challenge. These strategies exploit the timing and frequency of drug treatments to encourage the survival of some anthelmintic susceptible parasites that will give rise to L_3 as a means of reducing the proportion of resistant survivors. This inevitably compromises the short term effectiveness of GI nematode control, and a major challenge is to decide when to treat and which animals to treat or which to leave untreated. Aspects of the life history and reproductive biology of *Fasciola* inevitably constrain the impact of refugia based strategies in reducing selection for anthelmintic resistance, hence the only pragmatic yet unproven recommendations to reduce the emergence and spread of anthelmintic resistance are to avoid uderdosing and gene flow.

Poor livestock production is often blamed on suspicion of anthelmintic resistance, encouraging an approach with the simple objective of preventing anthelmintic resistance, rather than refining the complex aforementioned helminth control principles in particular livestock populations. This highlights the need for accurate diagnostic tools in the iterative development of effective helminth control programmes, to optimise livestock productivity and at the same time reduce or reverse selection for anthelmintic resistance, as defines sustainability

To-date, conventional studies of candidate genes related to the known methods of action of anthelmintic drugs have identified a causative association between three SNPs in the isotype 1 ß tubulin gene of GI nematodes and benzimidazole resistance, but have produced a complex picture for the other drug groups, implicating many genes. This highlights the challenges of working with these complex organisms, and emphasises the need for new approaches to robustly identify genetic markers associated with anthelmintic resistance. We have undertaken a series of genetic crosses between susceptible and multiple drug resistant strains of *Haemonchus contortus* to identify genomic regions linked to resistance genes. Distinct genetic crossing strategies were used: the first involved a novel backcross between either an African- or Australian-derived resistant strain and the ivermectin susceptible genome reference strain; and the second involved a conventional genetic cross between a north American-derived resistant strain and the susceptible genome reference strain, with subsequent drug selection of the progeny. We have used whole genome sequencing of the parental strains and output of the crosses, together with the high quality *H. contortus* genome assembly, to map drug resistance associated loci throughout the genome.

This talk will be based on our studies of gastrointestinal (GI) nematodes and *Fasciola* liver flukes in ruminant livestock as examples of broadly applicable principles. It will describe comparative genome wide analyses to identify the location of a major quantitative trait locus associated with ivermectin resistance in the same ~2 Mb region of chromosome V in three geographically and genetically divergent resistant *H. contortus* strains. Finally, it will outline our ongoing approach to map putative macrocyclic lactone resistance markers within this locus, and discuss how these might be applied to aid in the development of sustainable GI nematode control strategies.