Using RNA interference to investigate putative virulence factors of *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*

SAMUEL DUNCAN[1], ROBERT BRINZER[1], DANIEL PRICE[2], DAVID SMITH[2], TOM MCNEILLY[2], NICHOLAS ANDRONICOS[3], ALASDAIR NISBET[2], COLLETTE BRITTON[1]

[1] THE UNIVERSITY OF GLASGOW, SCHOOL OF BIODIVERSITY, ONE HEALTH AND VETERINARY MEDICINE, URQUHART BUILDING, GLASGOW G61 1QH, [2] MOREDUN RESEARCH INSTITUTE, PENTLANDS SCIENCE PARK, BUSH LOAN, PENICUIK, MIDLOTHIAN EH26 0PZ, [3] THE UNIVERSITY OF NEW ENGLAND, SCHOOL OF SCIENCE AND TECHNOLOGY, BIOCHEMISTRY AND IMMUNOLOGY, MCCLYMONT BUILDING, ARMIDALE NSW 2350, AUSTRALIA

The Trichostrongylid scour worms *Trichostrongylus* and *Teladorsagia* infect the gastrointestinal (GI) tract of their ruminant hosts, causing tissue damage and significant weight loss. Some livestock acquire protective immunity to scour worms, indicating that vaccination is a promising therapeutic approach. The precise mechanisms of immunity are poorly understood and optimisation of vaccine design and delivery are needed. Effective vaccine design requires knowledge of how scour worms establish infection and mediate host-parasite interactions. The ability to culture larval stages as they develop, together with the application of gene knockdown approaches such as RNA interference (RNAi), should aid vaccine design. We have developed a highly efficient exsheathment protocol and new growth media formulated from egg yolk that facilitates long-term culture of exsheathed L3 (xL3) of *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* to late L4 stage. Using this *in vitro* culture system, we optimised RNAi in both species. Soaking xL3 with double-stranded RNA (dsRNA) resulted in sustained transcript knockdown over 5 days, with up to 90% reduction for some target genes, with efficacy dependent on the specific target transcript. Our recent investigations showed that excretory/secretory (ES) proteins of *T. circumcincta* xL3 and L4 stage larvae induce expansion/swelling of ovine abomasal organoids. To identify the putative active protein(s) and mechanisms involved, size exclusion chromatography of ES was applied, and a fraction containing 12 proteins eliciting organoid expansion was identified. To discover which ES protein(s) mediate this expansion phenotype, RNAi knockdown of each target transcript was performed in xL3 *T. circumcincta*. RNAi-treated larvae were applied to ovine abomasal organoids and resulting expansion activity measured over 5 hours. Knockdown of two targets suppressed organoid expansion relative to control larvae. Further investigation to validate these as bona fide mediators of organoid expansion is ongoing. By future application of organoid and RNAi mutant co-culture models we aim to identify other scour worm genes involved in establishing a niche within the GI tract of their host, thereby validating parasite virulence factors as vaccine candidates *in vitro*.