Ruminating Over EVs: Microbiome Manipulation Through Rumen Fluke Extracellular Vesicles.

Leonard, J., Fisher, M., Cantecessi, C., Huws, S., Brophy, P. M. & Morphew, R. M.

Recent work has demonstrated a substantial contribution of parasite-mediated changes in the ruminant gut microbiota following investigation into the rumen fluke, *Calicophoron daubneyi*, within an *in vitro* rumen model. Within this setting, extracellular vesicles (EV) were identified as vital components in shaping bacterial communities within the host rumen, yet the direct effects are not fully understood. At present, EV release from helminths has only been demonstrated *in vitro* within helminth maintenance media. Thus, confirming EV release *in vivo* must be a priority. To assess this, EV populations were purified from 1) infected rumen fluid with a 4-hour incubation containing rumen fluke at 37^{o}C, 2) infected rumen fluid with no worm culture, 3) uninfected rumen fluid, and 4) DMEM with a 4-hour incubation containing rumen fluke at 37^{o}C representing an *in vitro* model. All samples were then centrifuged at 15,000 ×*g*, and filtered through 5 µm, 0.4 µm and 0.2 µm PTFE membrane filters. Following size exclusion chromatography (SEC) for the purification of rumen EVs, TEM has demonstrated the identification of small likely bacterial EVs in addition to larger EVs, which have only been identified within rumen fluke containing rumen fluids thus likely representing rumen fluke EVs. To confirm the larger EVs as *C. daubneyi* specific EVs, gold labelling TEM utilising fluke specific antibodies (Anti-FhGST-S1) known to bind to the surface of fluke EVs, will be used to identify EVs more accurately secreted into both *in vitro* rumen simulation and infected rumen fluid coupled with a meta-proteomics approach. In addition, utilising SEC purified *C. daubneyi* EVs in an optical density assay has identified antimicrobial and bacteriostatic activity. EVs derived from *C. daubneyi* cultured in DMEM, were observed to be more effective at suppressing *Escherichia coli* and *Bacillus megaterium* within these optical density assays when compared to EVs purified from *in vitro* rumen fluid cultivars. Future optical density assays aim to target additional rumen relevant microbes including *Prevotella, Ruminococcus* and *Staphylococcus*. Once these interactions are understood and characterised, novel approaches to control involving the interaction with the ruminant microbiome may be investigated.