Rumen Fluke-Microbiome interactions: Exploration into Understanding the Extracellular Vesicles of *Calicophoron daubneyi*

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

CALICOPHORON DAUBNEYI & EXTRACELLULAR VESICLES

The rumen fluke, *C. daubneyi,* has been found throughout Europe, inhabiting the same niche life cycle as the liver fluke *Fasciola hepatica*. Moving from Central Europe, *C. daubneyi* has begun to root itself firmly within the United Kingdom and the rest of Europe. Efforts to control *F. hepatica* has allowed *C. daubneyi* to proliferate through farms, through a lack of knowledge encompassing epidemiology and control options. Given the dearth of rapid diagnostics for rumen fluke the exact locations of the highest prevalence are still yet to be determined. Understanding the fundamental biology of *C. daubneyi* is now of paramount importance due to the emerging/increasing prevalence.

Extracellular vesicles (EVs) contain small packets of information, predominantly in the form of DNA, RNA, miRNA, tRNA and proteins, performing a variety of functions due to their ubiquitous nature. At present, research on rumen fluke EVs is limited to characterising the protein complement of EVs purified through differential centrifugation [1]. Given the close link between rumen fluke and the rumen microbiome it is perhaps not surprising that rumen fluke EVs may modulate the bacterial populations present in the rumen [1].

Thus, the aim of this research is to understand the role of helminth derived EVs on microbial populations to elucidate the potential mechanism behind microbial modulation. Initial focus will assess the antimicrobial activity of rumen fluke EVs followed by utilising metataxonomy and metaproteomics to identify the mechanisms and the effects of EVs in the rumen through *in vitro* fermentation models and experimental infections.

Figure1: Life Cycle of the Rumen Fluke *Calicophoron daubneyi*

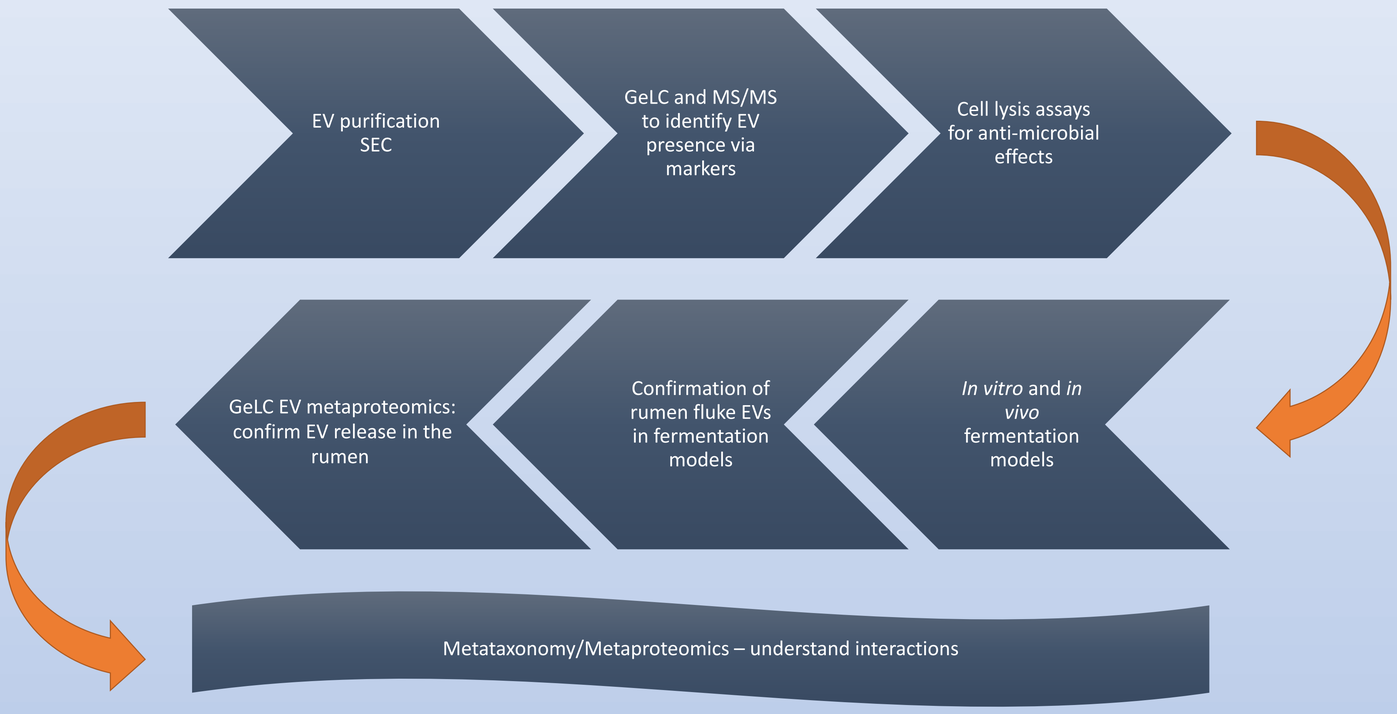

Figure 2: Workflow of the project.

APPROACH

Isolation and analysis of *C. daubneyi*'s rumen EVs: Size exclusion chromatography (SEC) has been used to isolate EVs from ES products, followed by confirmation using GeLC proteomics searching against the *C. daubneyi* transcriptome [2]. EV presence has been confirmed via EV marker identification. Functional assays: 1) Cell lysis assays 2) *In vitro* fermentation models to identify the presence of, and effect of, *C. daubneyi* EVs will be determined using SEC EV purification and GeLC based metaproteomics and metataxonomy. TEM will also aide in this confirmation using fluke EV specific marker antibodies, within the fermentation models, ascertaining how clean and efficient the models are. 3) Results will be compared to the EVs released within the rumen during *in vivo* infections. Metataxonomy will identify the microorganisms present during *C. daubneyi* infection, compared to the uninfected rumen microbiome.

RESULTS

Currently, EVs have been isolated from rumen fluke via SEC, and confirmed as EVs using GeLC and tandem mass spectrometry (Table 1). Cell lysis assays assessing EV antimicrobial activity are currently underway, which could pose fruitful due to the identification of lysosomal EV proteins such as cathepsin D [3]. The primary markers for EV identification are found within Vesiclepedia, for instance, beta actins [4]. With 324 total proteins hit, there was 183 proteins with a score >45, being significant, and suitable for analysis. Some non-significant similarities to other proteins have been identified, however these are moved forward for future analysis as to ascertain the function, this may also be a result of a new transcriptome which requires further annotation.

Table 1: BLASTx results of the twelve top MASCOT results for *C. daubneyi* EVs

TRANSCRIPTOME ACCESSION	PROTEIN FUNCTION	BLASTx ACCESSION
>TR19715|C0_G1_I1	Ezrin	KAF5404092.1| Ezrin-moesin-radixin [parazonium's heterotremus]
>TR9358|C0_G1_I1	Actin 7	XP_016695551.2| actin-7 [Gossypium hirsutum]
>TR25140|C0_G1_I1	Multidrug resistance-associated protein 1	TGZ64921.1| hypothetical protein CRM22_006127 [Opisthorchis felineus]
>TR24356|C0_G1_I2	PCD-6 Protein	KAA3672706.1| programmed cell death 6-interacting protein [Perigonium's Westermann]
>TR21569|C0_G5_I1	No Significant Similarities	No Significant Similarities
>TR25036|C3_G1_I11	Cathepsin D	THD19479.1| Cathepsin d lysosomal aspartyl protease [Fasciola hepatica]
>TR18939|C0_G1_I1	No Significant Similarities	No Significant Similarities
>TR25036|C3_G1_I3	Lysosomal Aspartic	XP_011196132.1| lysosomal aspartic protease-like [Zeugodacus cucurbitae]
>TR23254|C0_G1_I1	Leucine aminopeptidase	ABL11479.1| putative leucyl aminopeptidase [Clonorchis sinensis]
>TR17779|C0_G1_I1	Actin	XP_009173845.1| hypothetical protein T265_09499 [Opisthorchis viverrini]
>TR18070|C0_G1_I1	Actin Alpha/beta	THD26818.1|Actin alpha cardiac muscle 1 [Fasciola hepatica]
>TR24554|C0_G1_I1	Acid sphingomyelinase-like phosphodiesterase 3a	KAG5455085.1| Acid sphingomyelinase-like phosphodiesterase 3a [Clonorchis sinensis]

REFERENCES

[1] Allen, N. R., Taylor-Mew, A. R., Wilkinson, T. J., Huws, S., Phillips, H., Morphew, R. M., & Brophy, P. M. (2021). Modulation of Rumen Microbes Through Extracellular Vesicle Released by the Rumen Fluke Calicophoron daubneyi. Frontiers in Cellular and Infection Microbiology, 11. https://doi.org/10.3389/fcimb.2021.661830

[2] Huson, K. M., Morphew, R. M., Allen, N. R., Hegarty, M. J., Worgan, H. J., Girdwood, S. E., Jones, E. L., Phillips, H. C., Vickers, M., Swain, M., Smith, D., Kingston-Smith, A. H., & Brophy, P. M. (2018). Polyomic tools for an emerging livestock parasite, the rumen fluke Calicophoron daubneyi; identifying shifts in rumen functionality. Parasites & Vectors, 11(1), 617. https://doi.org/10.1186/s13071-018-3225-6

[3] Baechle, D., Flad, T., Cansier, A., Steffen, H., Schittek, B., Tolson, J., Herrmann, T., Dihazi, H., Beck, A., Mueller, G. A., Mueller, M., Stevanovic, S., Garbe, C., Mueller, C. A., & Kalbacher, H. (2006). Cathepsin D Is Present in Human Eccrine Sweat and Involved in the Postsecretory Processing of the Antimicrobial Peptide DCD-1L *. Journal of Biological Chemistry, 281(9), 5406–5415. https://doi.org/10.1074/JBC.M504670200

[4] Kalra, H., Simpson, R. J., Ji, H., Aikawa, E., Altevogt, P., Askenase, P., Bond, V. C., Borràs, F. E., Breakefield, X., Budnik, V., Buzas, E., Camussi, G., Clayton, A., Cocucci, E., Falcon-Perez, J. M., Gabrielsson, S., Gho, Y. S., Gupta, D., Harsha, H. C., … Mathivanan, S. (2012). Vesiclepedia: A Compendium for Extracellular Vesicles with Continuous Community Annotation. *PLOS Biology*, *10*(12), e1001450. https://doi.org/10.1371/JOURNAL.PBIO.1001450

ACKNOWLEDGEMENTS

The British Society of Parasitology for awarding the travel grant, and the conference. Food BioSystems DTP for supplying the funding, and training for the research. Aberystwyth University for the facilities, and training to conduct said research. Queen's University Belfast and the University of Cambridge for Co-supervision (SH and CC respectively). Ridgeway Research Ltd for CASE support (MF).