Ruminating Over Host-Parasite Interaction Models For Fluke Driven Immune Responses

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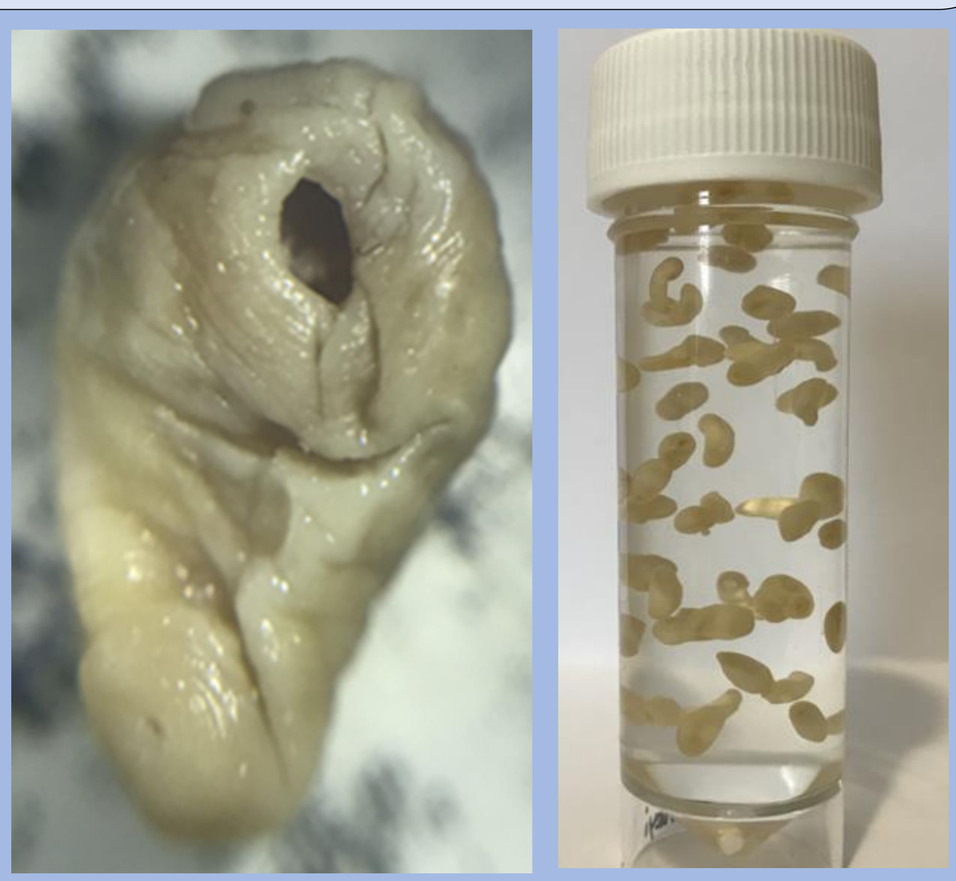
Background

Rumen fluke (RF), *Calicophoron daubneyi,* is a parasitic trematode of ruminant livestock which has rapidly increased in prevalence in the changing climate. Given this increase, further research is needed to understand how this parasite interacts with its host, especially in terms of the hosts immune response. Extracellular vesicles (EVs), contain predicted and confirmed immune modulators and are recognised as a key strategy of immunomodulation employed by parasitic helminths[1]. RF are demonstrated to produce EVs yet their actions on the hosts immune system is yet to be investigated[2].

Typically, *in vitro* cell culture models, have been used to investigate infectious diseases but such approaches cannot account for the multiple cell types present within tissues. As a result, there is growing interest in tissue explant models, whereby animal tissue are maintained *in vitro*. *In vitro* explants have been used to investigate many bacterial infectious disease but have not been used for helminth infections.

Aims: This study aims to initially establish an *in vitro* bovine ruminal explant model for assessing host immune responses and investigate the effects of adult RF EVs on the hosts immune system, using this model as a novel means of studying helminth infections.



Adult *Calicophoron daubneyi*

Approaches

Current Work

Establishment of Bovine Rumen Explant Model

An initial culture has been run to ensure the rumen tissue can mount an immune response and retains tissue architecture and viability.

Biopsies were dissected from the bovine rumen epithelial layer, inclusive of papillae, and cultured for up to 72 hours.

Rest vs No-Rest Period

Our previous work shows some tissue explants to mount an immune response in response to dissection of the tissue, thus require a 24hr rest prior to experimental stimulation.

This initial validation study has been run using a 24hr rest period vs no-rest period to assess whether bovine ruminal explants do or do not require resting prior to stimulation.

Assessment of tissue viability

RT-PCR of apoptosis gene markers to monitor cell death

Histology to look at tissue architecture

Assess the ability of the tissue to mount an immune response via stimulating with LPS to test a dose response

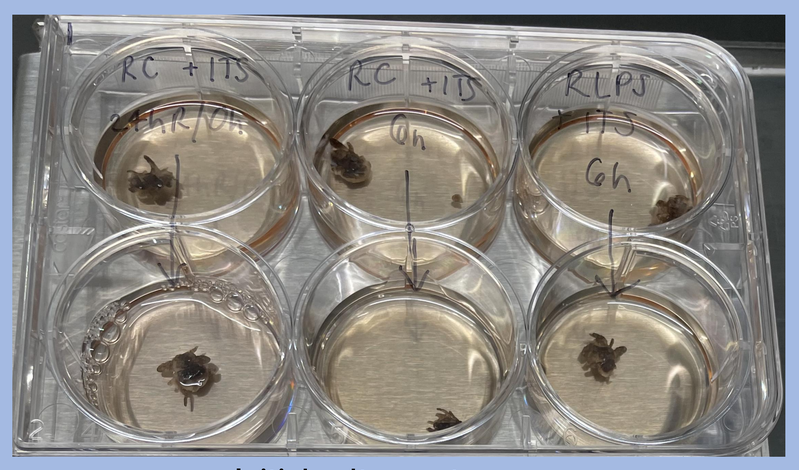
An immune response can be mounted to LPS in an ovine explant model but has not been tested in bovine rumen[3]. LPS is a bacterial antigen known to cause an immune response therefore is a good antigen to test if the tissue can mount a response prior to stimulating with parasite EVs.

Analysis of mRNA expression of immune related genes (E.g. IL4/IL5/IL13 involved in TH2 responses) at 6h

Protein secretion analysis at 24h

IL-6 ELISA to assess cytokine response

Sections of bovine rumen dissected away from muscular layer.



Initial culture set up.

Future Work

Stimulation of explant model with adult RF EVs.

- Purification and isolation of EVs from adult RF.
- Stimulation of rumen explant model with EVs.
- Assessment of tissue immune responses using cytokine secretion, transcriptome and proteome profiling.

Verification of rumen explant model.

- Stimulation of established bovine rumen epithelial cell culture model with adult RF EVs
- Assessment of immune response mounted by cells in response to adult RF EVs.
- Assessment of cell viability and metabolism in response to EV stimulation.

Stimulation of explant/cell culture models with immune modulator proteins expressed by adult RF.

- Synthesis of immune modulator proteins expressed by adult RF EVs.
- Assessment of cell and tissue immune response to synthesised proteins.

Assessment of immune parameters in bovine macrophages, a specialised immune cell designed to mount an immune response, in response to RF EVs.

- Stimulation of bovine macrophages with adult RF EVs.
- Assessment of changes in immune parameters over time to understand immediate and longer-term immune responses.

References

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