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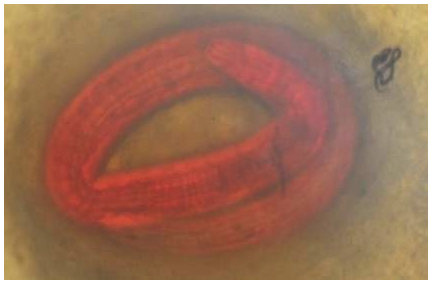
Evaluation of mast cell responses as a novel method to estimate equine cyathostomin burdens



The development of minimally invasive diagnostic tests to provide information on an individual's cyathostomin encysted larval burden has the potential to improve equine health and welfare

RS:243

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Mast Cell Recruitment and Activation as Measures of Cyathostomin Burden

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UK

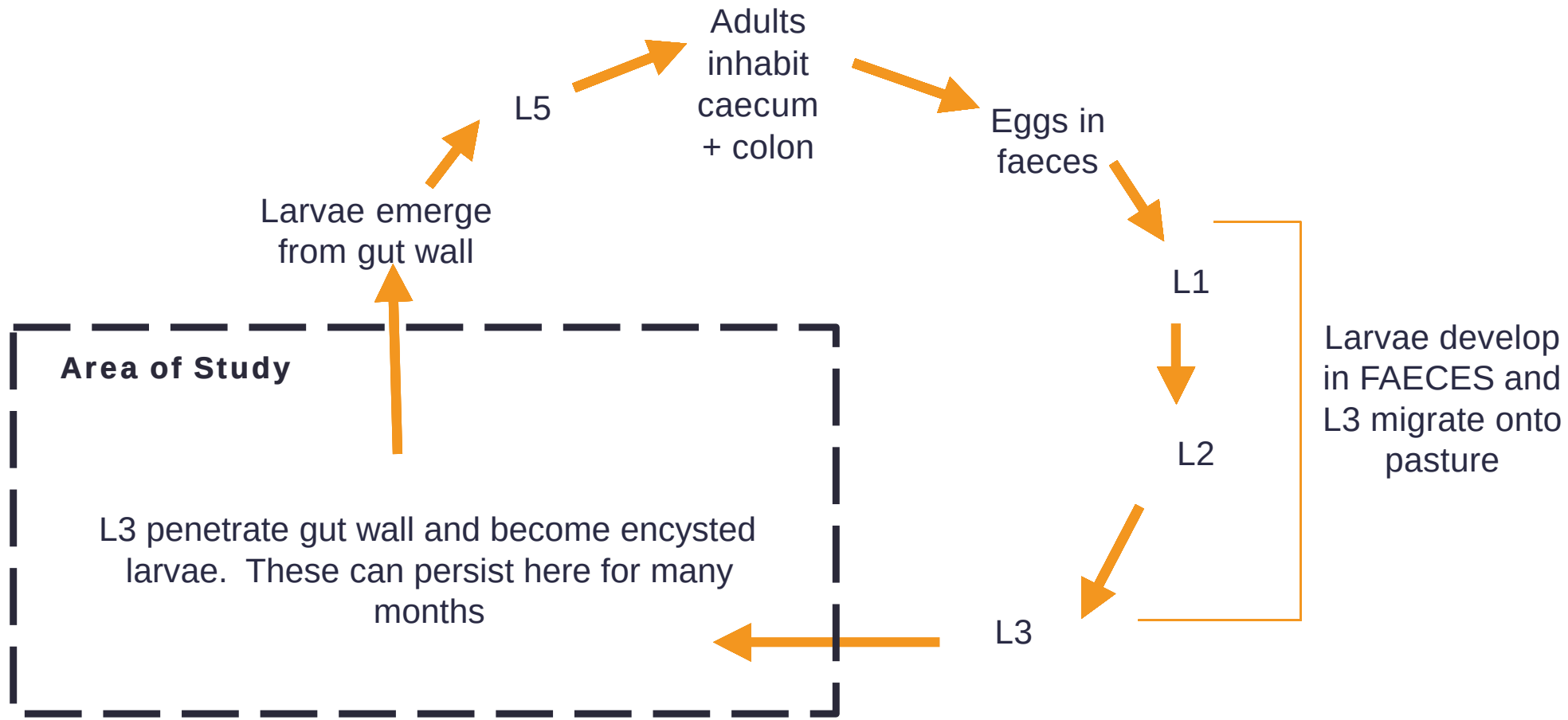
Dr Clements was awarded a PhD for the work described in this report

The Problem...

- Cyathostomins are the principal parasitic pathogens of horses
- Cyathostomins are highly prevalent worldwide and can affect all grazing horses
- Infection with these worms can lead to severe clinical signs
- Diagnosis is difficult due to a lack of specific tests for the immature stages of cyathostomins
- Resistance to dewormer drugs (anthelmintics) is a major threat and necessitates the delivery of evidence based (i.e. targeted) control measures

Cyathostomin lifecycle

This project focuses on encysted larvae which inhabit the gut wall

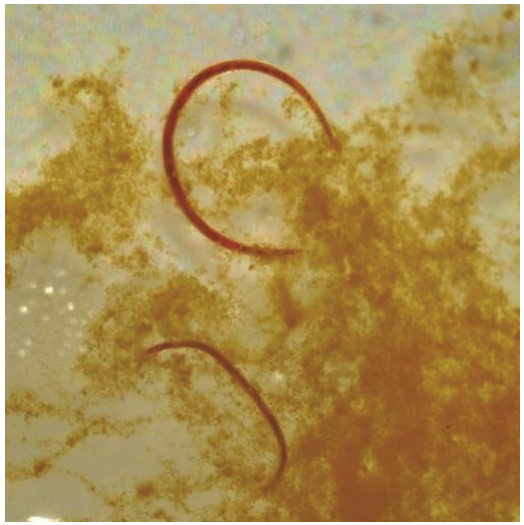


Encysted larvae

- The majority of infections are sub-clinical
- Clinical signs can be seen with high worm burdens
- Larvae can become inhibited in development for variable periods while encysted in the gut wall
- Mass emergence of encysted larvae can lead to larval cyathostominosis associated with
  - gut wall damage
  - weight loss, diarrhoea, colic, oedema, pyrexia
- Larval cyathostominosis carries a poor prognosis in some individuals

Anthelmintic resistance

- There is evidence of multi-drug resistance
  - with no reversion to sensitivity
- Sustainable control relies on faecal egg count (FEC) directed targeted treatments
  - FEC analysis assesses the output of adult female worms only
  - there is no method for measuring encysted larval burden
- Only some dewormers kill encysted larvae
  - as there are no methods for assessing larval burdens, current guidelines are to treat all horses with an appropriate ‘larvicidal’ treatment at certain times of year

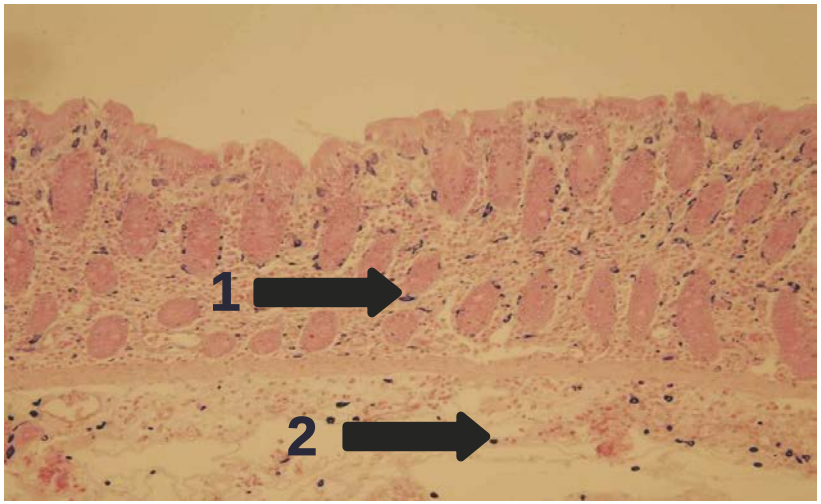


Mast cells

- Mast cells are a type of cell involved in immunity including protection against parasites
- There are two types; connective tissue and mucosal mast cells
- This project focussed on mucosal mast cells

There are two subsets of these cell types

1. Mucosal
2. Submucosal



Mast cells

- Increase in response to cyathostomin infection
- Produce serine proteinases - equine tryptase (eqTRYP) and equine mast cell proteinase-1 (eqMCP-1). These are:
  - major components of mast cell granules
  - found in different concentrations in different tissues
  - involved in mast cell mediated inflammatory responses in the gut
  - thought to be involved in tissue and vascular remodelling
- There is a strong positive relationship between cyathostomin burden and proteinase production in the caecum

How this project will benefit the Thoroughbred

- An improved understanding of intestinal responses to cyathostomin infection will help inform the development of minimally invasive diagnostic tests
- Such tests will
  - enable identification of horses at risk of severe disease
  - inform strategic dewormer targeting and so reduce pressure for resistance to anthelmintics
  - inform breeders/trainers/owners on an individual's infection status and hence provide information on its general health profile

Aims of this Study

Hypothesis One

- There is a positive association between mast cell numbers and cyathostomin larval burden throughout the large intestine
- Rectal biopsy may have diagnostic potential for estimation of larval burden

Hypothesis Two

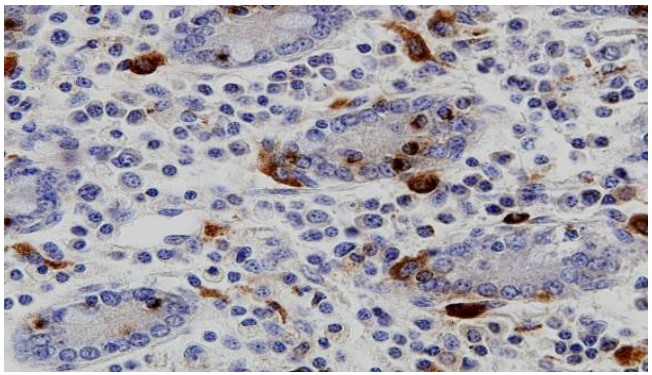
- Serum and tissue mast cell proteinase concentrations are positively associated with mast cell number and with cyathostomin larval burden
- Measurement of these will have diagnostic potential for prediction of burden

Hypothesis Three

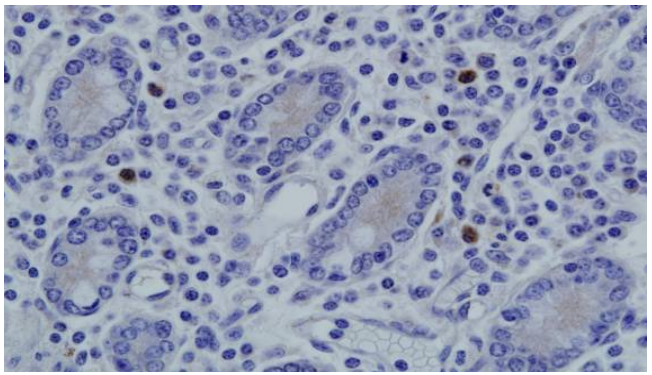
- Novel equine mast cell proteinase genes remain to be characterised and levels of proteins encoded by these may be associated with cyathostomin larval burden

Methodology

Immunohistochemistry protocols were optimised to allow visualisation of eqMCP-1 and eqTRYP expressing mast cells



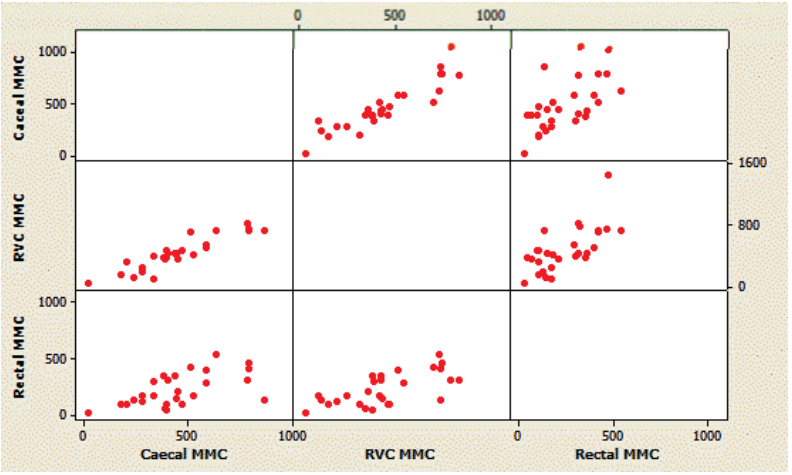
Anti eqMCP-1 labelled mast cells



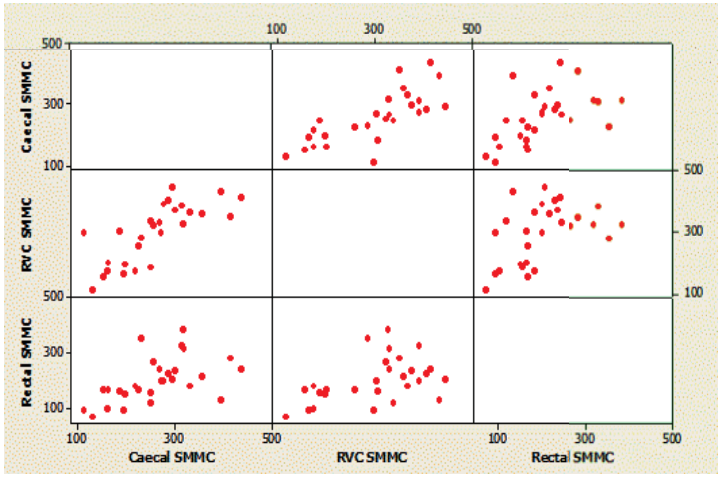
Anti eqTRYP labelled mast cells

A sandwich ELISA was optimised for detection of eqMCP-1 and eqTRYP. Local serum from blood vessels draining the intestinal tract and peripheral serum were tested here. Tissue homogenate from the caecum, RVC and rectum was prepared and also tested by ELISA

Testing hypothesis 1



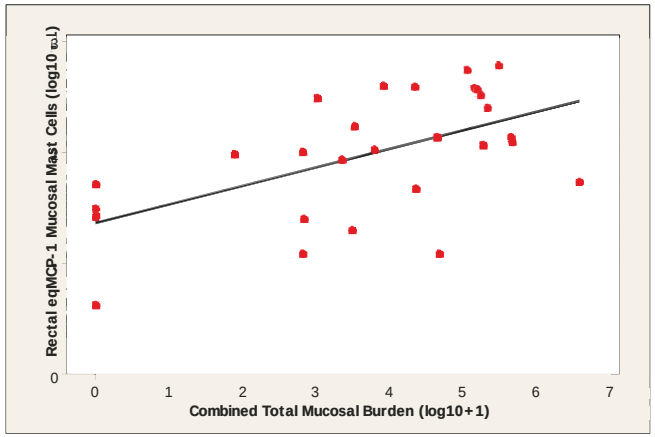
A



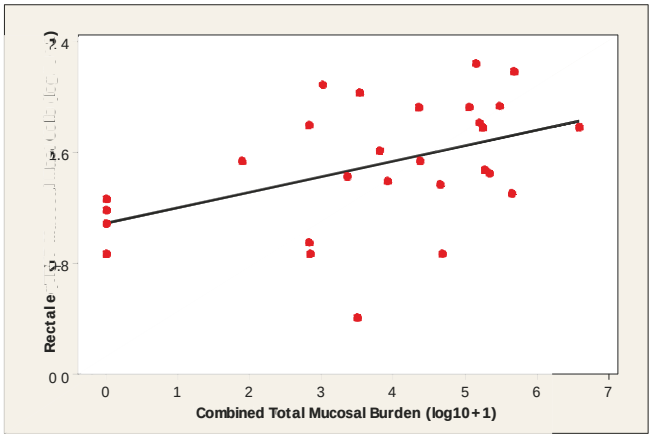
B

Mucosal (Figure A) and submucosal (Figure B) mast cells counts were significantly correlated between organs ($p<0.05$). (MMC: Mucosal Mast Cells. SMMC: Submucosal Mast Cells)

Testing hypothesis 1



A



B

There was a significant relationship between both rectal eqMCP-1 mast cell (Figure A: p=0.018, r2=43.1%) and rectal eqTRYP mast cell (Figure B: p=0.048, r2=24.41%) populations and the cyathostomin total mucosal burden (TMB)

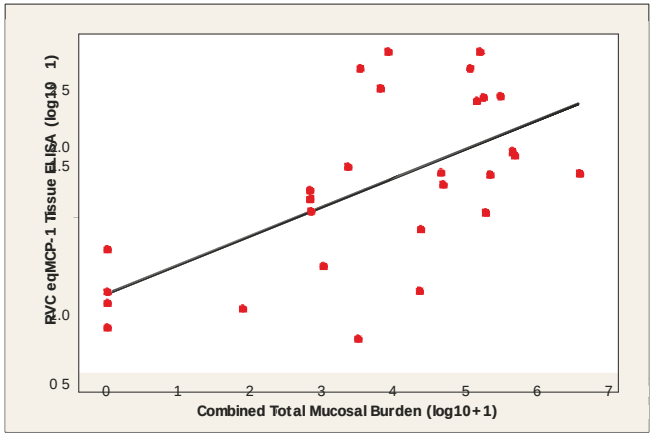
Testing hypothesis 2

ELISA analysis indicated that there was:

- no significant correlation between peripheral and local serum concentrations of eqMCP-1 ($p=0.203$, $rho=0.418$) and eqTRYP ($p=0.539$, $rho=-0.210$)
- a significant positive correlation between eqMCP-1 and eqTRYP local serum levels ($p=0.006$, $rho=0.665$)
- no significant correlation between serum eqMCP-1 and eqTRYP levels and mast cell number or proteinase expression ($p>0.05$)
- no significant relationship between serum eqMCP-1 and eqTRYP levels and cyathostomin TMB

Testing hypothesis 2

There was no significant relationship between tissue levels of eqMCP-1 or eqTRYP and cyathostomin TMB in either the caecum or rectum



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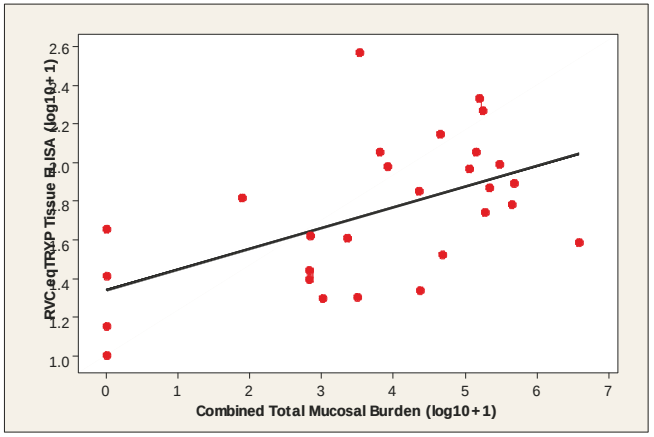


B

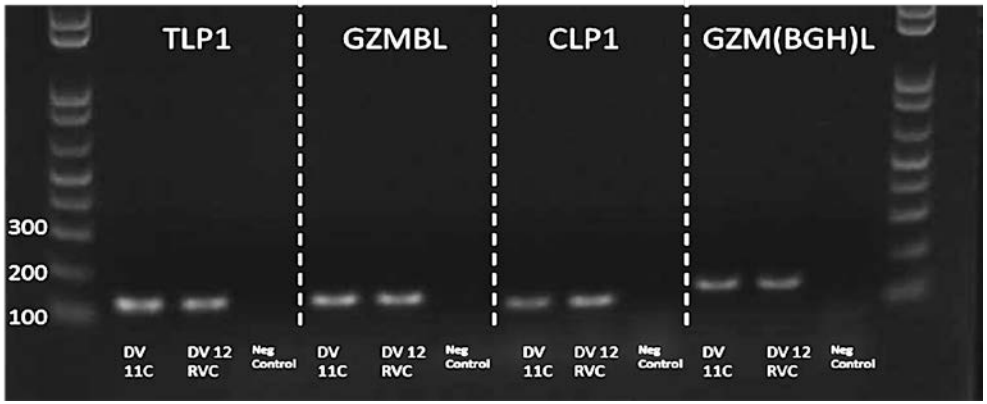
There was a significant relationship between tissue levels of eqMCP-1 (Figure A: $p=0.005$, $r^2=57.69$) and eqTRYP (Figure B: $p=0.023$, $r^2=38.08$) and cyathostomin TMB in the RVC

Testing hypothesis 3

Availability of the annotated horse genome enabled investigation into previously unpublished mast cell proteinase sequences. Four genes were selected for further analysis. These were:

- tryptase like proteinase 1 (TLP1)
- granzyme-B like proteinase (GZMBL)
- chymase like proteinase-1 (CLP1)
- granzyme(BGH)like proteinase-1 (GZMBGHL).

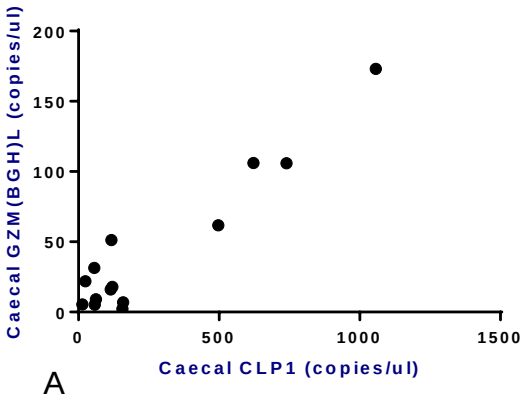
Transcription of the genes encoding these proteinases was confirmed in equine tissue using polymerase chain reaction (below).



Testing hypothesis 3

Levels of the four selected genes were assessed by quantitative PCR

- There was a significant positive correlation between levels of TLP1 and GZMBL in the caecum (Figure A), RVC and rectum, $p<0.05$
- There was a significant positive correlation between levels of CLP1 and GZM(BGH)L in the caecum (Figure B) and RVC, $p<0.05$
- There was a positive correlation between levels of CLP1 and GZM(BGH)L in the rectum approaching significance (Figure C: RB, $p=0.054$)



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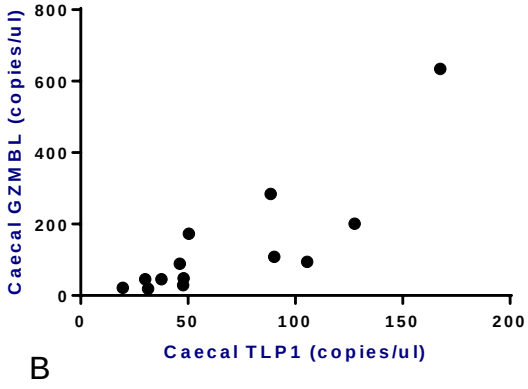

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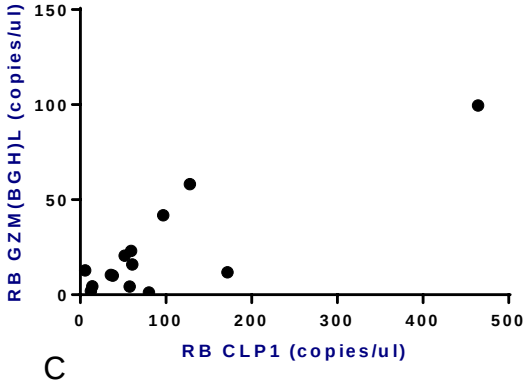
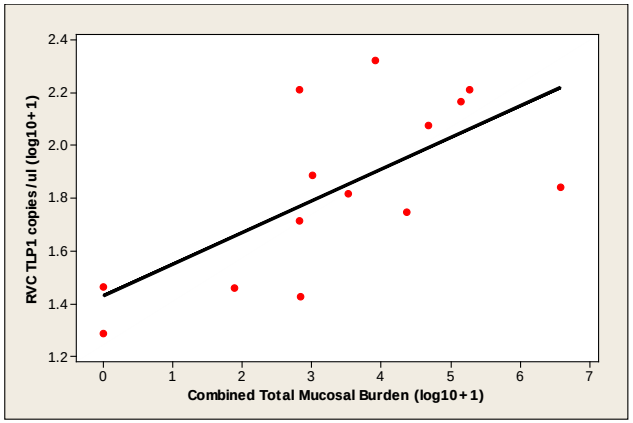
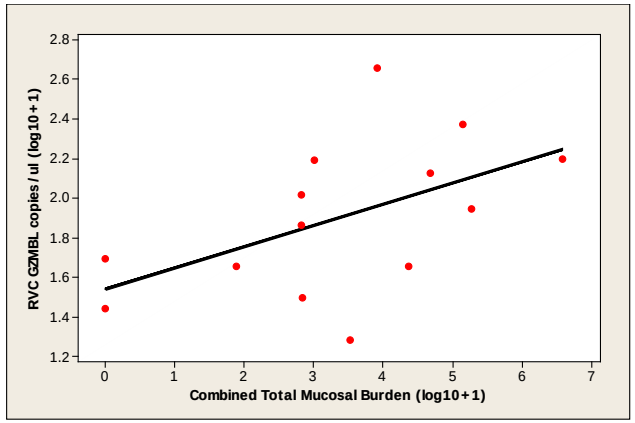


C

Testing hypothesis 3



A



B

- There was a significant positive relationship between both TLP1 (Figure A: $p=0.007$, $r^2=58.80$) and GZMBL (Figure B: $p=0.042$, $r^2=53.80$) expression and cyathostomin TMB in the RVC
- Relationships were not significant in the caecum or rectum, $p>0.05$

Conclusions

- Mast cell populations correlated throughout the intestine, providing evidence of the common mucosal system, whereby immune stimulation of one region may lead to activation of mucosal immune responses at sites distant
- EqMCP-1 and eqTRYP labelled cells were identified and there was a positive relationship between populations of these cells in the rectum and cyathostomin TMB
- There was no correlation between intestinal-derived and peripheral serum eqMCP-1 and eqTRYP concentrations, a limitation for using serum based diagnostics for this system
- There was a significant relationship between tissue levels of eqMCP-1 and eqTRYP and cyathostomin TMB in the RVC, however this was not observed in the caecum or rectum

Conclusions

- Four ‘new’ proteinase encoding genes were assessed using qPCR
- Transcript levels of TLP1 correlated with GZMBL levels. CLP1 and GZM(BGH)L levels also correlated. These proteinases could be produced by the same mast cell subsets or alternatively by other cells with production being induced by the same stimulation
- There was a significant positive relationship between TLP1 and GZMBL levels and cyathostomin TMB in the RVC, but not in the caecum or rectum
- A complex relationship exists between mast cells and cyathostomin burden. This may be affected by stage of infection, infection history, age, variation in pasture contamination, frequency of anthelmintic treatment, concurrent non-parasitic disease and nutrition status

Future Prospects

- Rectal biopsy studies will provide insight into the dynamics of mast cell responses and cyathostomin infections and the potential of sampling at this site for diagnostic tests

- Further exploration into associations between the wide range of equine mast cell proteinases and cyathostomins is warranted to investigate for enzymes that inform more specifically on levels of encysted larval infection

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Further reading

- Worming study – resistance to dewormer a serious health threat http://www.hblb.org.uk/documents/Racehorse_health/Worming%20study%20July13.pdf
- Anthelmintic efficacy on UK Thoroughbred stud farms - http://www.sciencedirect.com/science/article/pii/S0020751914000782

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