Horserace Betting Levy Board
5th Floor
21 Bloomsbury Street
London WC1B 3HF

Tel: 020 7333 0043
Fax: 020 7333 0041
Web: www.hblb.org.uk
Email: equine.grants@hblb.org.uk

Further development of a diagnostic blood test for larval stages of small redworm

VAC reporter C Marr

Professor Jacqueline Matthews

Prj: 756

Why do we need a test for larval cyathostomes?

- Immature cyathostomin larvae spend a large part of their lifespan in the gut wall. Here, they can cause severe disease, but cannot be detected. These immature stages are particularly important in young horses such as yearlings that graze pasture in large groups.

- This project made significant progress toward developing a diagnostic test, the function of which will be to estimate the burden of larvae the gut wall

- Availability of this test will enable identification of horses at risk of severe disease and will direct administration of appropriate dewormers. In so doing, the test will be used to inform strategic drug targeting and hence reduce selection pressure for dewormer resistance.

Background to this project

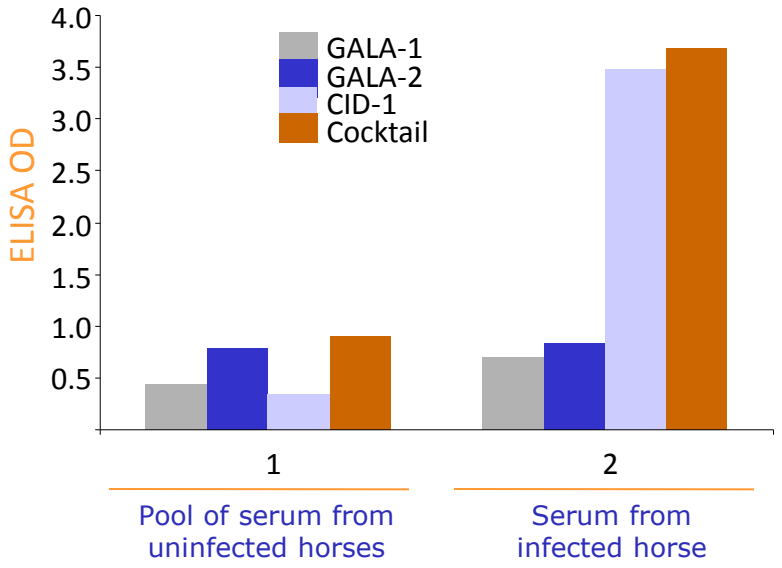
- This study builds on a series of projects which HBLB has funded on equine parasitology over the last decade
- This study was performed to further develop a diagnostic blood test for mucosal larval stages of the common small redworm
- All horses are susceptible to worm infections and heavy infection can cause loss of condition and serious disease, especially when large numbers of mucosal stage larvae are present
- This study is relevant to the Thoroughbred youngstock in particular because younger animals are most likely to be seriously affected by cyathostomes
- To find out more about our previous studies that explain the background to the current project, go to http://www.hblb.org.uk/documents/blog/Prj%20723%20Matthews%20FINAL.pdf

What is an ELISA?

- Also known as an Enzyme linked immunoassay, this is a technology that is widely used for detecting antibodies in human and animal plasma.
  - If there are antibodies, this means the individual has mounted an immune response to the micro-organism of interest.
  - The component of the micro-organism that the animal is produces antibodies against is called an antigen.
  - For the test to be successful, the antigen must be specific to the organism.
  - For a successful test for larval cyathostomes, we not only need an antigen that is only found in these worms but only present in larvae.
- The ELISA detects the presence of a molecules, for example specific antibodies, in a liquid sample like blood and the basis of the ELISA is
  - Molecule of interest are attached to a surface.
  - A specific antibody is applied over the surface so it can bind to this molecule.
  - This antibody is linked to an enzyme, and, after a variable number of steps, a substance containing the enzyme's substrate is added.
  - The reaction between the enzyme and the substrate produces a detectable signal, most commonly a colour change.

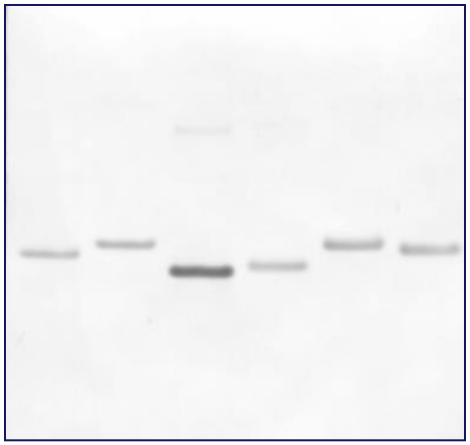
A cocktail of parasite proteins are promising:

- In our previous study, we found a "cocktail" of proteins, derived from the larvae, showed promise as the basis of an ELISA which could be used to distinguish infected and uninfected horses.



Aims & Objectives

- To build on previous work to develop a diagnostic blood test for larval cyathostominosis
- To increase test accuracy for all stages of cyathostomin larvae found in the large intestinal wall
- To increase test accuracy to take account of the multi-species nature of cyathostomin infections in horses

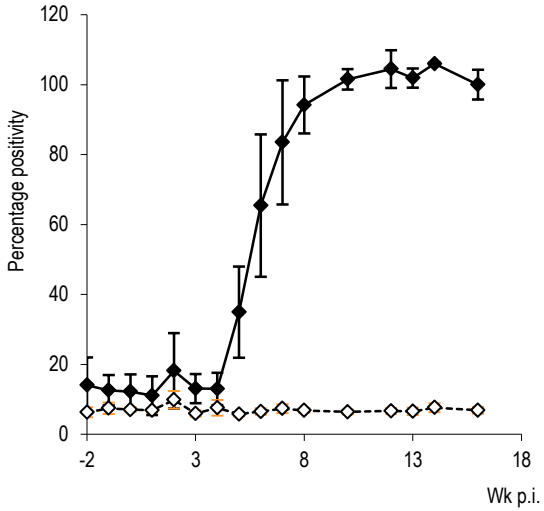


P A R C G L

We used immunoblot techniques to show that serum from clinical larval cyathostominosis cases to had antibody to the common species *C. pateratum* (P), *C. ashworthi* (A), *C. radiatus* (R), *C. calicatus* (C), *C. goldi* (G) and *C. longibursatus* (L). This confirms that multiple cyathostome species can infect a horse simultaneously.

What was achieved

- We confirmed that proteins selected for the test were present in all mucosal larval stages
  - early L3, late L3 and developing L4
- We ensured that common cyathostomin species were represented in the test by including proteins from these species
- We confirmed that the test was useful for diagnosis by investigating how serum antibody responses to the proteins, and cocktails of these indicated the levels of mucosal burden in individual horses

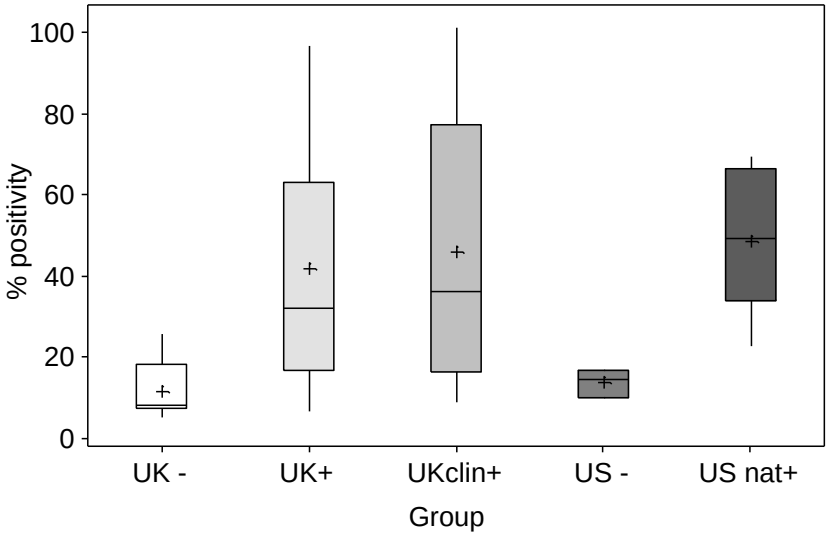


ELISA data: protein cocktail-specific serum IgG(T) responses of cyathostomin-infected ponies (unbroken line) *versus* uninfected ponies (dashed line) over a 20-wk time course

Main outcomes

- An early L3-specific protein was that was sufficiently immunogenic to inform on mucosal burden was not identified, but the main component of the test (GALA) was confirmed as being expressed in early L3 and its diagnostic value established in estimating burdens of these stages

- Cyathostomin species coverage of the test was widened to include 14 common species. The specificity and sensitivity of each protein component was confirmed.

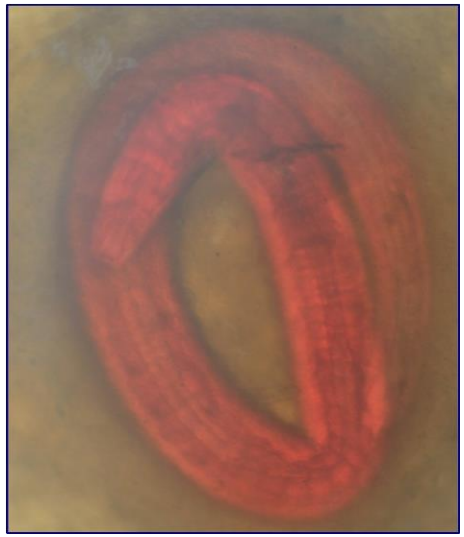


GALA cocktail tested in ELISA using blood samples from horses of known burden or from clinical cases. Samples came from the following: cyathostomin free horses from UK (UK-) and US (US-), cyathostomin infected horses from UK (UK+) and (USnat+), larval cyathostominosis cases (UKclin+). Data presented as % positivity compared with levels in infected ponies. Box plots for each group: mean = crosses, median = horizontal bar.

Next steps

- To finalise how the diagnostic test will be used in practice

- To commercialise the test so that it is available to veterinarians in the UK and abroad



Cyathostomin mucosal larva in large intestinal wall

To find out more about equine parasites:

J.B. Matthews, Facing the threat of equine parasitic disease

http://onlinelibrary.wiley.com/doi/10.1111/j.2042-3306.2010.00356.x/full