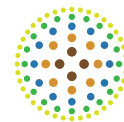


PROJECT SUMMARY



RURAL
INDUSTRIES

Research & Development
Corporation

Macrocyclic Lactone Resistance on Australian Horse Farms

Background

The horse industry relies heavily on macrocyclic lactone (ML) products such as ivermectin (IVM) for worm control. These are typically administered in an “interval treatment” regime (e.g. every 10-14 weeks). Such products are very effective against a broad range of parasites. However, both the heavy reliance on this one group of products, and the regular application regimes, are likely to increase selection for resistance among equine nematode parasites. No new class of anthelmintic has reached the equine market in over 25 years, and for this reason, the efficacy of existing drugs needed to be sustained for as long as possible.

Anthelmintic resistance is a growing concern for the international horse industry, particularly in light of the limited range of drug classes available for the treatment of worm infections in horses. In order to foster effective and sustainable worm control, we require a clear understanding of the efficacy of the drugs currently in use. Surveillance of drug efficacy against the important nematode parasites of Australian horses is incomplete.

The Faecal Egg Count Reduction Test (FECRT) has traditionally been used to monitor the emergence of anthelmintic resistance among veterinary parasites. However, interpretation of FECRT data in horses can be problematic because of the small numbers of horses on many properties (decreasing the statistical power of the assay), lack of validation of the technique with ascarid infections, and mixed cyathostomin infections being the norm. A recently developed molecular test (the Reverse Line Blot Hybridisation Test) has offered hope for differentiating the more common cyathostomin species, but has not been validated with Australian isolates.

In vitro tests such as the Egg Hatch Assay, the Larval Development Assay, and the Larval Migration Inhibition Assay provide alternative methods of assessing the susceptibility of parasites to drugs and have been used successfully in small ruminant drug resistance studies. Such assays have the potential to be more sensitive (able to detect resistance at an earlier stage) and less labour intensive, but unfortunately none of them have been validated for use with cyathostomins or *P. equorum*, in horses.



Aims and Objectives

The principal aim of this project was to investigate the current efficacy of ML drugs against eastern Australian populations of two important nematode parasites, cyathostomins and *Parascaris equorum*. The project also investigated the suitability of selected *in vitro* assays to identify variations in susceptibility to ML drugs between populations of these two parasites.





Methods used

- a. A standard FECRT was carried out on a total of 42 horse properties across eastern Australia to assess the efficacy of ML drugs, primarily Ivermectin (IVM), against cyathostomins. These properties were from south east Queensland, the Hunter Valley, Wagga Wagga and tropical northern Queensland. Properties were selected for inclusion in the study on the basis of numbers and ages of horses on each property. A further FECRT was carried out on a single group of foals to assess the efficacy of IVM against *P. equorum* on one property. Both the modified McMaster faecal egg counting technique and the Modified Wisconsin technique were utilised.
- b. Various modifications of the Larval Migration Inhibition Assay, which has been shown to successfully discriminate between susceptible and resistant populations of small ruminant nematodes, were investigated in an attempt to validate the LMIA for use with cyathostomins. Variations in incubation lengths and incubation temperatures, as well as different cyathostomin populations were assessed as part of this work.
- c. Preliminary work was carried out to adapt a published method for the in vitro culture of *Ascaris suum*, the pig ascarid, to *P. equorum*. This included evaluating methods for collecting or harvesting *P. equorum* eggs, as well as optimising culture and hatching conditions. This is the first line of investigation into the possibility of subjecting hatched *P. equorum* larvae to the LMIA or a similar in vitro assay.
- d. A published molecular assay, the Reverse Line Blot Hybridisation assay (RLBH), was investigated for application to Australian cyathostomin populations. If validated, this assay would allow investigators to analyse how drug treatment impacts upon selected species among the over 50 species of cyathostomins that may be found in horses.

Key Findings

In relation to ML efficacy against cyathostomins, the FECRT survey revealed that the ML drugs (primarily IVM) remained effective at 2 weeks post-treatment. In other words, this study provided no proof of ML-resistance among the study cyathostomin populations. However, additional data collected during the study period provided anecdotal evidence of a shortened egg reappearance period (ERP) on some properties, which can be interpreted as a shift in the sensitivity (towards resistance) of cyathostomins.

A FECRT conducted on one group of foals in southeast QLD showed reduced efficacy (just 65%) of IVM against *Parascaris equorum*, providing evidence for the presence of ML resistance in Australia.

The modified LMIA was able to measure a significant difference in LC50 between two cyathostomin populations, however, in the absence of a field-resistant isolate the relationship between LC50 and true resistance remains unproven. LC50 values were not consistent between assays conducted at different time points which suggests that the assay is best used for comparisons of larvae that are of a similar age (length of storage after harvest from the larval culture). Migration of cyathostomin larvae in control wells was substantially poorer than that achieved by small ruminant nematode larvae, but was able to be improved by increasing the incubation temperature during the assay. More optimization of the LMIA is required before it could be considered a reliable tool for the early detection of ML resistance in cyathostomins.

P. equorum eggs were able to be harvested, cultured and hatched in the laboratory using a modified protocol optimised for *A. suum*. There were significant challenges with these processes, culminating in a poor egg hatching rate and larval survival. Given the presence of such a low efficacy of ML drugs against *P. equorum* as measured by FECRT, this lengthy and laborious protocol cannot be justified in order to provide larvae for more sensitive in vitro resistance tests. As resistance begins to emerge to the Benzimidazole (BZ) drug class, there may be some potential to adapt alternative in vitro assays, such as the larval development assay or egg hatch assay to *P. equorum*, which would make use of some of the techniques evaluated in this project.

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(from previous)

Finally, the RLBH assay was partially validated on Australian cyathostomin isolates. The species-specific oligonucleotide probes used previously in overseas studies successfully hybridised to DNA from available Australian specimens, but multiple cases of cross-hybridisation were also observed which prevented the definitive identification of specific species present in a mixed batch of cyathostomins. Therefore, further optimisation of probe sequences to increase specificity is required before the RLBH assay could be used as a tool for the investigation of species composition in a pooled sample. The assay has greater potential for gaining insights into species-specific biology or drug sensitivity when applied to individual specimens rather than pooled samples.

Implications for relevant stakeholders

Since ML's are the cornerstone of equine worm control and make up the lion's share of registered anthelmintics, their continued efficacy is essential for the future of parasite management for horses. The broad survey of ML-resistance among cyathostomins yielding no evidence of reduced efficacy at 2 weeks post treatment, therefore, provides encouraging news for the horse industry. ML's should continue to be utilised as part of an evidence-based, targeted, strategic worm control program.

A reduction in the ERP of cyathostomins following treatment with ML's, which has been documented overseas, appears to be occurring in Australia too. This finding has far-reaching implications, given that reduced ERPs often precede the emergence of drug resistance. This emphasizes the need for good product stewardship and also regular monitoring of drug efficacy.

The poor efficacy of IVM against *P. equorum* reported in this study is of concern. ML drugs form the foundation for most worm control programs, and *P. equorum* is the most pathogenic of the nematodes infecting weanlings, impacting on growth rates and occasionally causing small intestinal obstructions. With continued use of ML's in foals and weanlings, the prevalence of ML-resistance in populations of this worm can be expected to rise. This, combined with the additional observation of a shortened egg reappearance period for cyathostomins following ML treatment emphasises the need for prompt revision of the current worm control strategies of horse breeders, owners and managers.

The emergence of an accurate and sensitive alternative to the FECRT for assessing anthelmintic resistance of cyathostomins or *P. equorum* remains out of reach at this time, requiring more work by researchers.





Recommendations

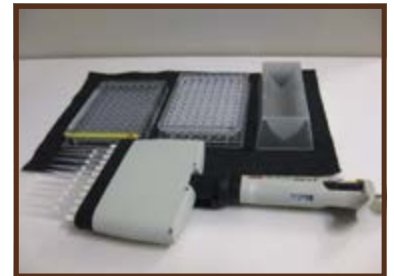
For horse owners:

- Whilst MLs currently show a high level of efficacy against cyathostomins, monitoring of the efficacy of MLs (and other drug classes) against cyathostomins and *P. equorum* remains important and should be carried out regularly via FECRT to ensure adequate control is being achieved.
- ML drugs, if shown to be ineffective, should not be used in isolation for control of *P. equorum* in foals and weanlings. Either a BZ drug or alternatively a combination product containing a non-ML active ingredient should be used. Where an ML product is used, FECRT to monitor the efficacy of treatment is recommended.
- Addressing the shortening of ERP of cyathostomins requires that the frequency with which ML drugs are administered to horses should be reduced where possible. FEC's should be utilised as a tool to enable the timing and frequency of treatment to be customised to individual horses or groups of horses. As an extension of the FECRT, ERP's should be monitored on a routine basis.



For researchers:

- The LMIA requires further optimisation before it can be considered a useful tool in detecting early ML resistance in cyathostomins. Future work: maximising larval migration in control wells, investigation of effect of larval batch age on LC50, protocol standardisation and inter-laboratory testing.
- Further work to optimise the in vitro culture of *P. equorum* for the purpose of producing larvae suitable for use in a LMIA is unwarranted. Future efforts should be directed toward testing for BZ-resistance in this parasite.
- Use of the RLBH assay to assess cyathostomin species composition before and after ML treatment in pools of eggs/larvae was not supported by this study. Future work should be directed toward improving the specificity of DNA probes. Validation against positive control specimens from different geographical regions is also required. A first step in achieving this outcome would be to collect a vast number of morphologically identified cyathostomin individuals to create a DNA library which could be shared with other researchers, and vice versa.



For more information

Anne Beasley, School of Veterinary Science

The University of Queensland, Gatton Campus, QLD, 4343

Phone: 07 5460 1968 **Email:** a.beasley@uq.edu.au

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