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A survey of macrocyclic lactone efficacy in Australian cyathostomin populations

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ABSTRACT

The macrocyclic lactone (ML) drugs are central to the control of equine strongyles but recent international reports raise concerns about reduced efficacy of these drugs against cyathostomins. The objectives of the present study were firstly, to evaluate the efficacy of ML drugs against cyathostomins on a cross-section of Australian horse farms, and secondly, to determine the egg reappearance period (ERP) following treatment of horses with MLs. A total of 419 horses on 43 properties were treated orally with ivermectin, abamectin or moxidectin, at recommended dose rates and drug efficacy was determined using the faecal egg count reduction test. Efficacy of 100% at 14 days post-treatment was reported on all of the 43 farms. ERP following ivermectin treatment was 6 weeks on two properties and ERP following moxidectin treatment was 12 weeks on a third property. These ERPs are shorter than those reported at the time of commercial release of these drugs which likely reflects changing drug susceptibility of the cyathostomin populations tested. Ongoing surveillance of drug efficacy and ERPs should be part of an integrated management approach to equine worm control that prioritises the preservation of anthelmintic efficacy.

1. Introduction

Due to favourable climatic conditions in many regions (particularly coastal regions) of Australia, cyathostomin worms present an ongoing threat to horse health. Although generally less pathogenic than large strongyles, cyathostomins can cause significant disease. In addition to the larval cyathostominosis syndrome associated with mass emergence of encysted larvae, characterised by diarrhoea and weight loss, cyathostomins have also been associated with anorexia and colic (Love et al., 1999). Given the impact of these parasites, there is a need to manage the use of effective anthelmintics to delay the emergence and spread of drug resistance (Matthews, 2014).

Drugs available to treat cyathostomins in Australia include benzimidazoles (BZs), macrocyclic lactones (MLs) and tetrahydropyrimidines (THPs). Suppressive treatment programs throughout the 1960′s, 70′s and 80′s used BZs to target *Strongylus vulgaris* and other large strongyles but led to widespread BZ-resistance among cyathostomin populations (Kaplan and Vidyashankar, 2012). BZ-based products, sometimes in combination with other drug groups such as THPs, remain widely available in Australia. ML drugs, including ivermectin (IVM), abamectin (ABM) and moxidectin (MOX), currently constitute > 50% of the equine anthelmintic products registered in Australia (Public Chemical Registration Information System Search, Australian Pesticides and Veterinary Medicines Authority). MOX has a prolonged anthelmintic effect compared to IVM and ABM, and greater efficacy against mucosal stages of cyathostomins (Bairden et al., 2006). The third drug class for worm control in equines, the THPs, includes pyrantel and morantel. Both are included in a range of combination anthelmintic products with either MLs or BZs, however, neither are currently available as single-active products. This very limited selection of effective chemical treatments for cyathostomins, and the apparent absence of new drug classes under development for the equine market, underlines the importance of sustaining the efficacy of the ML drugs.

An essential part of managing ML resistance is proactively monitoring drug efficacy, a practice advised but seldom undertaken on Australian properties. The emergence of ML resistance among cyathostomins was predicted almost two decades ago by Sangster (1999), but reports of resistance, as measured by the faecal egg count reduction test (FECRT), are still scarce in the literature. Since the first suspected case was reported from the UK following treatment of donkeys with MOX (Trawford et al., 2005), reports from Brazil (Molento et al., 2008; Canever et al., 2013), the UK (Relf et al., 2014), Italy (Traversa et al., 2009), Germany (Von Samson-Himmelstjerna et al., 2005) and New Zealand (Bishop et al., 2014) have demonstrated a reduced efficacy of

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MLs against cyathostomins in FECRTs. More common are reports of shortened egg reappearance periods (ERPs) (von Samson-Himmelstjerna et al., 2007; Dudeney et al., 2008; Molento et al., 2008; Lyons et al., 2008b; Rossano et al., 2010; Lyons et al., 2011; Lyons and Tolliver, 2013), and these are considered evidence of a shift in ML-sensitivity toward resistance. Critical tests have shown a reduced IVM efficacy of only 0–16% against luminal L4 stages (Lyons et al., 2009; Lyons et al., 2010; Lyons and Tolliver, 2013), thereby shortening the time taken for egg shedding to recommence following treatment.

There are comparatively few investigative reports into anthelmintic resistance among cyathostomins on Australian horse properties. Pook et al. (2002) reported a 100% FECR following treatment of horses with IVM on 7 NSW properties ($n \geq 4$ horses), there has been only a single Australian case report of suspected ML resistance involving an individual horse (Edward and Hoffmann, 2008). In that case, monthly faecal egg counts (FECs) were performed on a 24-year-old horse that was treated 7 times within a 13-month period using various ML worming products. Although FECs were not performed within the recommended time frame of 10–14 days post-treatment, as per FECRT guidelines, the study most notably reported a count of > 2000 epg 3–4 weeks following one IVM treatment and an increase from 200 to 500 epg 3 weeks after a MOX treatment. FEC results of another two horses on the same property were not discussed in detail and the authors reported no evidence of reduced efficacy on 9 other properties investigated. Without further investigations, this case should be considered as anecdotal evidence only.

Australian contributions to the global dataset on anthelmintic efficacy against equine helminth populations are scarce. We report here the results of a large survey of ML efficacy across a broad cross-section of Australian equine properties.

2. Materials and methods

2.1. Participating farms

A variety of strategies were used to purposively recruit equine properties. Participants were recruited based on their ability to provide a minimum of 10 horses for pre-treatment testing and willingness to perform collections from individual horses on 2 separate occasions. Publicly listed horse studs or stables were contacted directly. Articles were published in equine magazines and newsletters calling for suitable participants, and social media was also utilised to inform horse owners of the study and call for suitable participants. A total of 43 properties were recruited to participate in the study during 2012 and 2013, and were located in regions across four Australian States (Queensland $n = 23$ properties, New South Wales $n = 13$, Victoria $n = 4$, and South Australia $n = 3$). The number of horses on each farm included in the FECRT ranged from 6 to 15 (median 10) and the total number of horses examined in the study was 419. Age data was provided for 322 of the 419 samples. Age of horses ranged from 3 months up to 37 years of age (mean = 8.8 years, median = 7.0 years). Animal ethics approval for the project was granted by the University of Queensland's animal ethics committee (approval number ANFRA/148/12).

Three of the recruited QLD properties (referred to as properties A, B and C) were selected to also undergo an extended period of FEC testing to measure ERP. These properties were all located in the same region of southeast QLD and were selected based on their managers' willingness to comply with the extended weekly sampling protocol. Properties A, B and C differed in their anthelmintic treatment histories and the ages of horses (Table 1).

2.2. Faecal egg count reduction test and determination of egg reappearance period

The FECRT was carried out in accordance with the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for horses (Coles et al., 1992). FECs were performed using a modified McMaster technique with a lower detection limit of < 10 epg (range 6.2–7.5 epg) and a Whitlock Universal Slide (J A Whitlock & Co., Eastwood, Australia).

On day 0, pre-treatment samples were collected from all available horses prior to administration of a commercial equine anthelmintic containing an active ingredient from the ML class (ivermectin, 0.2 mg/kg, 29 properties; abamectin, 0.2 mg/kg, 10 properties; or moxidectin, 0.4 mg/kg, 4 properties). Samples were sealed in air-tight bags (expelling as much air as possible) and submitted to the laboratory by express post (maximum transit time = 3 days). We acknowledge that without vacuum packaging, truly anaerobic conditions cannot be created and some egg development may have been possible during transit (Nielsen et al., 2010; Sengupta et al., 2016). Post-treatment samples were collected on day 14 only from horses with a pre-treatment FEC of ≥ 150 epg. FECR (%) for each property was calculated according to American Association of Equine Practitioners (AAEP) Parasite Control Guidelines (Nielsen et al., 2016). Using the equation below, the FECR (%) for each horse in the group was calculated individually.

Formula used to calculate FECR (%):

$[(FECpre - FECpost)/FECpre] \times 100$.

The mean reduction for all horses tested was then calculated to determine the percent reduction for the property. While equine-specific criteria are yet to be established to define the presence of ML-resistance, the AAEP Parasite Control Guidelines (Nielsen et al., 2016) recommend using a reduction of mean faecal egg count < 95%. These guidelines are in line with the recommendations of the WAAVP (Coles et al., 1992) for FECRTs in sheep, which include a supporting criterion of a lower confidence limit (LCL) of < 90%.

On three farms, further sampling was undertaken during spring to determine the ERP following treatment with either MOX (1 farm) or IVM (2 farms). Faecal samples were collected from individual horses weekly from 2 weeks post-treatment until the ERP was reached. Faecal sampling ceased at 7, 8 and 12 weeks post-treatment for properties A, B and C, respectively. ERP was defined as the period of time from treatment until the FECR (%) was ≤ 90% (von Samson-Himmelstjerna et al., 2007; Larsen et al., 2011; Nielsen et al., 2016; van Doorn et al., 2014).

Comparisons among pre-treatment FEC of properties A, B and C were done using one way ANOVA (Kruskal-Wallis Test with Dunn's multiple comparison) (Graphpad Prism v6.05).

3. Results

The distribution of pre-treatment FECs was overdispersed and strongly skewed to the right (Fig. 1), in line with what is typically observed in parasite count data (Crofton, 1971; Sreter et al., 1994; Galvani, 2003; Scheuerle et al., 2016).

The 419 FEC values had a minimum of 150 epg, a median of 524 epg, a maximum of 4269 epg, and a mean ± SE of 723 ± 33 epg. Only 17% of the values were < 200 epg.

The mean percent reduction in FEC at 14 days post-treatment on all 42 properties surveyed was 100% following treatment with IVM, ABM or MOX.

On the three properties where ERP was measured, pre-treatment FECs ranged from 236 to 1740 epg (mean 667 epg, 95% CI 489–845, $n = 6$) for property A, 181–2282 epg (mean 1107 epg, 95% CI 704–1510, $n = 18$) for property B, and 359–918 epg (mean 741 epg, 95% CI 491–991, $n = 6$) for property C (Fig. 2), and were not significantly different ($P = 0.2356$).

For the IVM treatment properties (A and B), strongylid eggs were first detected in weeks 5 and 4, respectively, and the FECR threshold of 90% was reached between weeks 5 and 6 post-treatment for both properties, indicating an ERP of 6 weeks. For the MOX treatment group (property C), strongylid eggs were first detected at week 9 and the FECR was 90.4% at week 11, and had dropped to 81% by week 12 (Table 2),