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Anthelmintic therapy of equine cyathostomin nematodes – larvicidal efficacy, egg reappearance period, and drug resistance

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ABSTRACT

Cyathostomins are ubiquitous in grazing horses across the world, and anthelmintic resistance has been reported with increasing levels over past decades. The aims of the present study were (i) to investigate the efficacy against encysted larval stages of moxidectin (0.4 mg/kg) and fenbendazole (10 mg/kg daily for five consecutive days) and compare these regimens at 2 and 5 weeks post-treatment, (ii) to investigate individual cyathostomin species associated with shortened egg reappearance periods, and (iii) to document species exhibiting decreased susceptibility to the evaluated compounds. Thirty-six ponies were allocated to treatment groups with half euthanatized 2 weeks post-treatment, and the remainder necropsied after 5 weeks. Luminal and mucosal worm counts were conducted and strongyle egg counts were determined at weekly intervals. At 2 weeks, mean reductions of early L3s were 50.4% and 73.8% for fenbendazole and moxidectin, respectively. At 5 weeks, the respective efficacies were 51.3% and 71.8%. Two week efficacies against late L3s and L4s (LL3s/L4s) were 70.8% and 74.6% for fenbendazole and moxidectin, respectively, whereas very low numbers were found in all three groups at 5 weeks. None of the mucosal counts were significantly different between treatment groups. Fenbendazole and moxidectin reduced luminal worm counts by 93.2% and 98.3% at 2 weeks following administration, with moxidectin group adult counts being significantly lower than the other two groups ($P < 0.0001$). Both treatment groups had increased counts 3 weeks later ($P = 0.0415$). A moxidectin ERP of 4 weeks was associated with surviving luminal L4s, and adult species contributing to this were *Cyathostomum catinatum*, *Cylicostephanus longibursatus*, *Cylicocyclus ashworthi* and *Cylicocyclus nassatus*. This study documented (i) larvicidal efficacy of fenbendazole much lower than historical standards, (ii) survival of luminal immatures (L4) following moxidectin administration, and (iii) new information about cyathostomin species associated with these phenomena.

1. Introduction

Cyathostomin infection is widespread in horses across the world, with prevalence rates commonly reaching 100%. While the vast majority of horses remain asymptomatic, cyathostomins are capable of causing pronounced disease. A defined syndrome known as larval cyathostominosis has been described, with a case-fatality rate of approximately 50% (Reid et al., 1995). This condition is caused by the synchronous emergence of encysted cyathostomin larvae from the mucosa of the large intestine. Cyathostomin larvae are known to undergo arrested development at the early L3 stage (EL3) and can accumulate in populations that may reach hundreds of thousands (Eysker et al., 1984). Subsequent larval stages, including late L3 (LL3) and L4 stages, develop before the larvae leave their cysts and enter the intestinal lumen. This process has been associated with a pronounced inflammatory reaction and, when large numbers of larvae emerge synchronously, it can cause severe typhlocolitis and a protein-losing enteropathy (Love et al., 1999).

Only two currently marketed equine anthelmintic drugs have label claims for efficacy against encysted cyathostomins: moxidectin gel (MOX, 0.4 mg/kg) administered once orally, and fenbendazole (FBZ, 7.5 or 10 mg/kg) administered orally once daily for five consecutive days. It is well documented that anthelmintic resistance of cyathostomins to the adulticidal dose of FBZ is widespread (Kaplan et al., 2004; Lester et al., 2013). Despite general acceptance that FBZ may no longer have satisfactory adulticidal efficacy, many

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practitioners continue to recommend an annual larvicidal treatment as a distinct element of parasite management programmes. Furthermore, strongyle egg reappearance periods (ERP) have decreased to only 4–5 weeks following MOX treatment (Rossano et al., 2010; Lyons et al., 2011; Relf et al., 2014). This is a marked reduction compared with the 16–22 weeks initially reported for this anthelmintic drug (Jacobs et al., 1995; Demeulenaere et al., 1997; DiPietro et al., 1997). Recent observations suggest that a shortened ERP is caused by resistant luminal L4 (immature) stages which survive treatment and quickly mature into egg-laying adults (Lyons et al., 2009, 2010; Lyons and Tolliver, 2013). It is currently unknown if these shortened ERPs can be associated with a loss of mucosal larvicidal efficacy as well. We recently completed a study comparing the larvicidal efficacies of MOX and the 5 day FBZ regimen against a population of cyathostomins known to be resistant to the adulticidal, single dose of FBZ (5 mg/kg) (Reinemeyer et al., 2015). Necropsies with total worm counts were conducted at 14 days after completion of treatment, and the larvicidal efficacies of MOX and FBZ were 85.2% and 71.2% against LL3/L4 mucosal larvae and 63.6% and 38.6% against EL3 stages, respectively. While larvicidal efficacy of MOX was within the expected range, the efficacy of FBZ was well below initially reported efficacy levels (Xiao et al., 1994; Monahan et al., 1995, 1996).

While the prior study provided useful evidence, the post-treatment interval was too brief to evaluate the ERP of MOX. Furthermore, it is still unknown whether necropsy at 14 days post-treatment is the optimal interval for determining larvicidal efficacy. Because MOX is a very lipophilic drug that accumulates in fat tissue (Lanussi et al., 1997; Craven et al., 2002), it might exert cumulative larvicidal effects over time. Most published studies have evaluated the larvicidal efficacy of MOX at 14 days post-treatment, and larvicidal efficacies have ranged between ~50% and 100% (Xiao et al., 1994; Monahan et al., 1995, 1996). It has been proposed that the short interval between treatment and necropsy might be insufficient to allow larval elimination from all tissues (Bairden et al., 2006). Similar arguments can be made for FBZ, although this drug does not share the same lipophilic properties. Even in the absence of efficacy, it is possible that a prolonged interval after treatment would confound accurate interpretation of larvicidal activity. Current knowledge of the population dynamics of cyathostomin nematodes indicates that therapeutic removal of adult and L4 worms from the lumen of the gut may stimulate the more mature encysted stages (LL3 and L4) to complete development and emerge into the gut lumen to repopulate the vacated adult niche (Eysker et al., 1989). Accordingly, EL3s could also resume development, and would be present as LL3s or L4s at 5 weeks post-treatment. Under this scenario, the absence of EL3s could be interpreted as a stage-specific larvicidal effect, whereas it might just be the culmination of normal population dynamics following adulticidal therapy. In the absence of a direct comparison, it remains unknown whether larvicidal efficacy estimates would differ if necropsy were performed at 2 or 5 weeks post-treatment.

The aim of this study was to compare the larvicidal efficacies of MOX and FBZ at two intervals, 2 and 5 weeks post-treatment, in a population of research ponies. Secondary aims were to measure the ERP following MOX treatment, and document the species composition of worms surviving either treatment and contributing to post-treatment egg production.

2. Materials and methods

This study, conducted between September 25 and October 10, 2015, was approved by the University of Kentucky's Institutional Animal Care and Use Committee (IACUC), USA, under protocol number 2015–2092.

Thirty-six mixed-breed ponies between 2 and 4 years of age were enrolled and kept on pasture for the duration of the study. The ponies were naturally infected with cyathostomin parasites and none of them had received any anthelmintic treatments for at least 12 months prior to the study. In 2014, they were each treated once with either MOX (individuals born in 2013) or ivermectin (IVM) (individuals born in 2011 or 2012). Prior to that, the population was treated up to four times per year with either IVM, MOX or FBZ. Anthelmintic efficacy against cyathostomin parasites harboured by these ponies was unknown prior to this study. Individual strongyle fecal egg counts (FECs) were performed using the mini-FLOTAC method with a detection limit of five eggs per gram (EPG) of feces (Barda et al., 2014). The ponies were blocked by age and ranked by decreasing magnitude of egg counts. Each three consecutively ranked ponies comprised a replicate, and each of the three ponies within a replicate was randomly allocated to one of three treatment groups: MOX, FBZ or untreated control. Thus, each treatment group comprised 12 ponies. Half the ponies enrolled in each group were euthanatized and necropsied at 2 weeks post-treatment, and the remainder were necropsied at 5 weeks. All study personnel were blinded to group allocation and treatment assignments throughout the study.

All ponies were kept on the same pasture for the duration of the study. On Day −1, individual body weights were measured with a certified livestock scale and anthelmintic doses were prepared based on body weight and label dosages. Ponies in the FBZ group were treated orally with Panacur PowerPak (10 mg/kg for 5 days, Merck Animal Health, Madison, NJ, USA) once daily on Days 0–4. Ponies in the MOX group were treated orally with Quest (400 μg/kg, Zoetis, Kalamazoo, MI, USA) once on Day 4. Ponies assigned to the control group remained untreated.

Fecal samples were collected from all ponies on Days 11 and 18, corresponding to 7 and 14 days after treatment completion. FECs were performed in triplicate with the mini-FLOTAC method, using a saturated glucose-salt solution (specific gravity of 1.26) as flotation medium. FEC reductions (FECRs) were calculated for all ponies on Days 7 and 14, and group means with 95% confidence intervals were determined for each time point. On Days 18 and 19, three randomly selected ponies from each group were euthanized and necropsied concurrently for determination of total worm counts. Thus, six complete replicates were processed ~2 weeks post-treatment.

The remaining 18 ponies, comprising another six complete replicates, remained enrolled for an additional 3 weeks. FECs for this remaining cohort were performed with fecal samples collected on Days 21, 28 and 35. Group means were determined for each time point, and FECRs were calculated for Days 21, 28 and 35. On Days 35 and 36, three ponies from each group were euthanased and necropsied for determination of total worm counts. Thus, six complete replicates were processed ~5 weeks post-treatment.

2.1. Enumeration of luminal cyathostomins

At necropsy of each pony, 2% aliquots of alimentary contents were collected from each compartment of the large intestine, cecum, ventral colon and dorsal colon, and preserved with an equal volume of 10% formalin. Small volumes of preserved contents were examined microscopically at 40 and 100× magnification, and cyathostomin specimens were recovered, enumerated and preserved in 10% formalin for later identification to species. Cyathostomin subtotals for each large intestinal compartment were calculated as the number of cyathostomins recovered divided by 0.02. Luminal treatment efficacies were calculated as the mean percent reduction relative to the corresponding untreated control group ((control − treatment)/control). Efficacies were estimated