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Research paper

Combination deworming for the control of double-resistant cyathostomin parasites – short and long term consequences

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ABSTRACT

Equine cyathostomin are pervasive gastrointestinal parasites with wide-spread resistance to the benzimidazole and tetrahydropyrimidine drug classes worldwide. Combination deworming has been proposed as a more sustainable parasite control strategy. Simulation studies have found combination deworming to be effective in controlling drug resistant ovine trichostrongylid parasites. One equine study demonstrated an additive effect of a combination of oxibendazole and pyrantel pamoate against cyathostomins. However, this is the only equine study evaluating combination therapy, and the effects of repeated combination treatments administered over time remain unknown. The purpose of this study was to observe the efficacy of repeated oxibendazole/pyrantel pamoate combination therapy administered over one year against a cyathostomin population with resistance to benzimidazole and pyrantel products. Fecal egg counts were determined for the entire herd (N = 21) at the day of anthelmintic treatment and at two-week intervals for eight weeks post treatment. Starting efficacies of oxibendazole (OBZ, 10 mg/kg) and pyrantel pamoate (PYR, 6.6 mg base/kg) were 66.7% and 63.3%, respectively. Hereafter, the herd was treated four times with an oxibendazole/pyrantel pamoate combination, eight weeks apart, followed by repeating the single active treatments before concluding the study. While the first combination treatment exhibited an additive effect of the two active ingredients, this efficacy was not sustained over the course of the study. Mean fecal egg count reduction (FECR) was significantly greater for the first combination treatment (76.6%) than the second (42.6%, $p = 0.0454$), third (41.6%, $p = 0.0318$), and fourth (40.7%, $p = 0.0372$) combination treatments. The final single active mean FECRs were 42.3% for oxibendazole, and 42.7% for pyrantel pamoate. These efficacies were not significantly different from the initial single active efficacies (OBZ, p = 0.4421; PYR, p = 0.8361). These results suggest that combination therapy against double resistant equine cyathostomin populations is not sustainable, when using actives with markedly decreased starting efficacies.

1. Introduction

Cyathostomins are clinically important helminth parasites of the horse, and typically comprise 99–100% of the total worm burden (Nielsen et al., 2010). The early third larval stage (EL3) can enter a hypobiotic state as they encyst into the mucosal lining of the large intestine (Eysker et al., 1984). Most horses do not exhibit signs of infection, however, the disease larval cyathostominosis may occur in rare cases. Simultaneous excystment of larvae from the mucosal lining of the cecum and colon can result in an array of clinical signs, including weight loss, diarrhea, dehydration, subcutaneous edema, and pyrexia (Love et al., 1999; Peregrine et al., 2006). The disease has been reported fatal in 50% of diagnosed cases (Reid et al., 1995).

Presently, there are three anthelmintic drug classes approved for controlling equine helminth parasites; the benzimidazoles, the tetrahydropyrimidines, and the avermectin/milbemycins (also known as macrocyclic lactones). Originally, parasite control regimens were based on frequent treatments with benzimidazole drugs, and development of additional drug classes resulted in the rotation between drug classes (reviewed by Kaplan and Nielsen, 2010; Nielsen, 2012). A proposed benefit warranting rotational deworming methods was to avoid overexposure of a single drug-class to a parasite population in hopes of preventing anthelmintic resistance (Prichard et al., 1980), but this has not proved to be a sustainable approach. Unfortunately, the frequent use of anthelmintics has driven the development of anthelmintic resistance in cyathostomins. Resistance to the benzimidazole and tetrahydropyrimidine drug classes is reported world-wide, and there are increasing reports of shortened egg reappearance periods and decreased

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efficacy following treatment with the macrocylic lactones (reviewed by Peregrine et al., 2014). Furthermore, some cyathostomin populations are observed harboring multi-drug resistance (reviewed by Peregrine et al., 2014).

It was originally proposed that genes associated with anthelmintic resistance likely occurred at very low levels in a naïve parasite population, and therefore may be associated with an ecological fitness disadvantage (Prichard, 1990). Under the selection pressure of anthelmintic use, however, these genetic mutations would offer an advantage and worms surviving treatment allow for the resistance alleles to increase in frequency within the parasite population (Prichard, 1990). Recent work has established that anthelmintic resistance may arise in a population in one of four ways; (1) pre-existing alleles are present prior to anthelmintic exposure, (2) spontaneous mutations occur immediately before or at the time of anthelmintic exposure, (3) frequent mutation events may allow alleles to appear recurrently, or (4) resistant alleles may have arisen elsewhere and were brought into the population through host migration (Gilleard and Beech, 2007). Gastrointestinal nematodes of small ruminants, and likely horses as well, are presumed able to acquire resistance at such a high rate because of their high fecundity and ability to undergo rapid rates of nucleotide sequence evolution, contributing to a high level of genetic diversity (Blouin et al., 1995; Anderson et al., 1998; reviewed by Gilleard, 2013). The trichostrongylid nematodes of small ruminants, *Haemonchus contortus* and *Teladorsagia circumcincta*, have been the most widely studied species due to their high infection prevalence and the extremely high levels and rates of resistance to multiple anthelmintic actives. A recent study regarding the emergence of anthelmintic resistance among populations of these species supports the latter two theories mentioned above. This model proposes that resistance occurs from multiple independent mutations recurrently arising and are spread by host migration (Redman et al., 2015).

It is interesting to note that even in the absence of a selection pressure, resistant alleles appear to remain within a cyathostomin population. Lyons et al. (2007) reported sustained resistance in a benzimidazole resistant cyathostomin population after remaining unexposed to anthelmintic treatment for 22 years. Another cyathostomin population, known as Population S, developed resistance to the benzimidazole drug class over a 18 year period from repeated use of cambendazole for four years (Drudge et al., 1983) followed by treatment with oxibendazole for 14 years (Drudge et al., 1985a,b; Lyons et al., 1994). Lyons et al., 2001 reported that after the subsequent seven years, in which pyrantel pamoate was used and pyrantel resistance was documented. The resistance to the benzimidazole drug class was unaffected despite the change in drug class use.

Computer modelling studies suggest that combination deworming, defined as using different drug actives to target the same parasite, may preserve drug efficacy and slow the development of resistance (Smith, 1990; Barnes et al., 1995; Leathwick, 2012). Leathwick (2012) used a computer model to observe the effects of combining a new active with an active to which resistance existed. They found the development of resistance to the new active to be delayed when used in combination, but this effect was decreased in scenarios with lower starting efficacies and in populations with low parasite refugia. Leathwick (2013) performed another modelling study to observe the rate of resistance development during 40 years of selection when sheep were treated with two drugs used in either annual rotation or in combination. Overall, the rate of resistance development was slowed when the drugs were used in combination. These results suggest that a combination of actives may increase the probability of killing parasites harboring the genetics for resistance to either one of the actives used. Furthermore, field studies performed in lambs infected with drug resistant trichostrongylids found a combination of multiple actives to have an additive effect (Bartley et al., 2004, 2005; Entrocasso et al., 2008; Le Jambre et al., 2010). Combination deworming has been found to be most successful when resistance does not exist to either of the drug classes used. Modelling studies performed by Barnes et al. (1995) and Leathwick (2012) found that combining drugs, when one or both are 100% effective, slows the rate of resistance. Once resistant alleles become prevalent in a population, however, this strategy is unlikely to be successful. Even with low levels of resistance, it is presumed that combining multiple actives may result in a synergistic effect (reviewed by Fleming et al., 2006). However, efficacy is unlikely to be beneficial once high frequency of resistant alleles to both actives are present (reviewed by Fleming et al., 2006; reviewed by Bartram et al., 2012).Presently, combination treatments are increasingly recommended to combat anthelmintic resistance in nematodes infecting ruminants (Bartram et al., 2012; Geary et al., 2012; Ramos et al., 2016) and against equine nematodes (Scott et al., 2015). Combination products are currently marketed in New Zealand, Australia and South America. However, the lack of effective anthelmintic drug classes available for equine cyathotomins questions whether combining actives would be effective against these parasites. To date, only one equine study has been performed evaluating combination therapy against cyathostomins. The results illustrated an additive effect against drug resistant cyathostomins after a single treatment with a combination of oxibendazole and pyrantel pamoate (Kaplan et al., 2014).

Presently, it is unknown how repeated combination treatments will affect a cyathostomin population harboring double-drug resistance. The aims of this study were (1) to evaluate the combined drug efficacy of oxibendazole and pyrantel pamoate for treatment of a herd naturally infected with a cyathostomin population with known drug resistance to both actives; (2) to observe changes in the efficacies of the single actives after four repeated combination treatments; (3) to test the additive effect formula proposed by Bartram et al. (2012) for estimating the efficacy of a combination treatment; and (4) to characterize the strongyle population before and after treatment using coprocultures.

2. Materials and methods

2.1. Ponies

A band of 21 Shetland ponies housed at the University of Kentucky was used in this study. The herd consisted of 20 mares and 1 stallion, ranging from ages 3–20 years. The herd harbors a population of cyathostomin parasites with documented resistance to the benzimidazole and tetrahydropyrimidine drug classes, otherwise known as Population S (Lyons, 2003). The ponies are maintained outside year-round. During the warmer months (March to October), the ponies were kept in dry lot with restricted access to striped grazing and were provided grass hay, consisting of either timothy or orchard grass. During the winter months, the ponies continued to receive hay and also had access to pasture which was comprised of clover, blue grass, and an assortment of weeds. Salt and mineral blocks were available ad libitum. The research was conducted under the approval from the University of Kentucky's Institutional Animal Care and Use Committee (IACUC) under protocol number 2012-1046.

2.2. Study design

This study took place between April 2015 and June 2016. All ponies were weighed on an electronic scale prior to each treatment and treated at 110% of their body weight every eight weeks. The 110% dosage was used to account for any drug loss that may have occurred during or following drug administration. Ponies were ranked by pre-treatment fecal egg count (FEC) and blocked into groups of two. Within each block, ponies were randomly assigned to a single active treatment of either oxibendazole (OBZ) or pyrantel pamoate (PYR) for the first treatment. Fecal egg counts (FEC) were determined at the day of treatment and every two weeks post-treatment. Eight weeks later, the single active treatments were repeated with the groups reversed. Following this, all ponies received four combination treatments with