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Comparison of the larvicidal efficacies of moxidectin or a five-day regimen of fenbendazole in horses harboring cyathostomin populations resistant to the adulticidal dosage of fenbendazole

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ABSTRACT

Despite widespread acknowledgement of cyathostomin resistance to adult icidal dosages of benzimidazole (BZD) anthelmintics, many strongyle control programs continue to feature regularly scheduled larvicidal treatment with fenbendazole (FBZ). However, no studies have been conducted to evaluate the efficacy of larvicidal regimens against encysted cyathostomins in a BZD-resistant (BZD-R) population. A masked, randomized, controlled clinical study was conducted with 18 juvenile horses harboring populations of cyathostomins that were considered BZD-R on the basis of fecal egg count reduction (FECR). Horses were blocked by prior history, ranked by egg counts, and allocated randomly to one of three treatment groups: 1—control, 2—FBZ >10 mg/kg once daily for five consecutive days, or 3—moxidectin (MOX) >0.4 mg/kg once. Fecal samples were collected prior to treatment and seven and 14 days after the final dose of anthelmintic. On Days 18–20, complete replicates of horses were euthanized and necropsied, and 1% aliquots of large intestinal contents were recovered for determination of complete worm counts. The cecum and ventral colon were weighed, and measured proportions of the respective organ walls were processed for quantitation and characterization of encysted cyathostomin populations. The five-day regimen of FBZ achieved 44.6% fecal egg count reduction, had 56.4% activity against luminal adults and larvae, and was 38.6% and 71.2% effective against encysted early third stage (EL3) and late third stage/fourth stage (LL3/L4) cyathostomin larvae, respectively. In contrast, MOX provided 99.9% FECR, removed 99.8% of luminal stages, and exhibited 63.6% and 85.2% efficacy against EL3 and LL3/L4 mucosal cyathostomins, respectively. Although BZD-R was the most feasible explanation for the lower larvicidal efficacies of FBZ, mean larval counts of moxidectin-treated horses were not significantly different from controls or those treated with FBZ. The lack of significant differences between larvicidal treatments was partially attributed to a small sample size and high variability among worm burdens. Historical differences in the time intervals between treatment and necropsy were identified as a confounding factor for accurate estimation of larvicidal efficacy. Determining appropriate post-treatment intervals for measuring larvicidal efficacy remains a critical regulatory and scientific challenge for this therapeutic area.

1. Introduction

Cyathostomin nematodes are ubiquitous parasites of grazing horses world-wide. Although adult worms in the intestinal lumen are considered fairly benign, synchronous emergence of cyathostomin larvae encysted within the mucosa of the cecum and colon causes a disease syndrome known as larval cyathostominosis. Larval cyathostominosis is a protein-losing enteropathy characterized by generalized typhlocolitis, profuse, watery diarrhea, passage of cyathostomin larvae in the feces, and a guarded prognosis for survival (Love et al., 1999). Historically, accumulation of large numbers of encysted larvae has been averted through anthelmintic or management interventions implemented to limit environmental contamination with strongyle eggs.

The effectiveness of this general approach has been compromised in recent decades, however, by cyathostomin resistance to various classes of anthelmintics (Peregrine et al., 2014). Resistance to benzimidazoles (BZD) is the most prevalent example in managed horse populations (Kaplan et al., 2004; Traversa et al., 2009; Lester et al., 2013), and the adulticidal dosage of fenbendazole (FBZ;

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5 mg/kg) often fails to achieve ≥90% reduction of strongylid egg counts. Even five-day regimens of FBZ at higher daily dosages of 7.5 mg/kg (Chandler et al., 2000) or 10 mg/kg (Rossano et al., 2010; Mason et al., 2014) have reportedly failed to achieve acceptable egg count reduction.

Only two currently-marketed equine anthelmintics have approved label claims against encysted cyathostomin larvae: moxidectin gel (MOX; 0.4 mg/kg) administered once orally, and FBZ (10 mg/kg) administered orally once daily for five consecutive days (Duncan et al., 1998 Xiao et al., 1994). Despite universal acknowledgement of cyathostomin resistance to adulticidal dosages of FBZ, some horse owners and veterinarians apparently assume that a five-day regimen at a higher dosage can overwhelm BZD resistance, based on the continued inclusion of larvicidal FBZ regimens as a regular or annual feature of a comprehensive parasite control program. However, no studies have been conducted to evaluate the efficacy of larvicidal regimens against encysted cyathostomins in a known, benzimidazole-resistant (BZD-R) population. A controlled trial was conducted to generate evidence to address this clinical question.

2. Materials and methods

2.1. Animals

Twenty juvenile horses (12 fillies; eight geldings), 12–24 months of age, were selected from three resident groups with differing anthelmintic histories. One group had received no anthelmintic treatment for at least seven months. The second and third groups had been treated with ivermectin (0.2 mg/kg) seven months previously. In addition, the third group had received daily pyrantel tartrate until five weeks prior to enrollment in the present study. All candidates were maintained on infective pasture until the start of acclimation.

A fecal sample from each candidate horse was examined quantitatively, and those with strongylid egg counts >125 eggs per gram (EPG) were treated orally with an adulticidal dose of FBZ paste (5 mg/kg). Fecal samples were collected from the same horses 14 days post-treatment, and fecal egg count reduction (FECR) was calculated for each. Horses which exhibited <80% FECR were considered to be harboring BZD-resistant cyathostomin populations, and were eligible for enrollment. Qualified candidates were housed in individual stalls beginning on 13 February, 2014 and acclimated to study conditions for seven days.

2.2. Experimental design

A masked, randomized, controlled clinical study was conducted in juvenile horses at a single site to evaluate the efficacies of a five-day regimen of FBZ oral paste (10 mg/kg) or a single, oral treatment with MOX gel (0.4 mg/kg) against luminal and mucosal stages of a putative, BZD-R cyathostomin population.

Qualified candidates were blocked by prior anthelmintic history, ranked within block by decreasing magnitude of strongylid egg count, and allocated randomly to one of three groups. After animals had been assigned to numbered groups, one of three treatments was allocated randomly to each group number. Group 1 horses served as untreated controls. Group 2 horses were treated once daily on Days 0 through 4 with doses of Safeguard paste (Merck Animal Health, Summit, NJ, USA) measured to provide ≥10 mg FBZ/kg body weight. Group 3 horses were treated once on Day 4 with a dose of Quest Gel (Zoetis Inc., Kalamazoo, MI, USA) measured to provide ≥0.4 mg MOX/kg body weight. Individual doses of both anthelmintics were prepared by transferring weighed quantities of either formulation to labeled dispensing syringes. Syringes were weighed after dosing to confirm that appropriate quantities of anthelmintic had been delivered to the intended recipient.

Fecal samples were collected seven and 14 days after the final dose of anthelmintic (*i.e.*, on Days 11 and 18), and FECR percentages were calculated. On Days 18–20, complete replicates of horses were euthanatized and necropsied for determination of total worm counts. Equal numbers of animals from each treatment group were processed on the same day.

Masking was achieved by complete separation of duties. Personnel who determined outcome measures (clinical observations, body weights, egg counts, worm counts) had no knowledge of individual treatment assignments. Similarly, unmasked personnel who prepared and administered doses of FBZ or MOX did not determine any outcome measures.

2.3. Animal care

This study was approved by East Tennessee Clinical Research's Institutional Animal Care and Use Committee, protocol number ETCR-14-0134.

General health observations of all study animals were conducted once daily for the duration of the study (Days −7 to 20). Observations included an assessment of general health, behavior/attitude, fecal consistency, and appetite. A physical examination of each candidate was conducted on Day −1.

Horses were maintained in individual stalls from Days −7 to 20 (final day of necropsy). Enrolled horses were fed a commercial equine concentrate (11% protein) in measured quantities totaling 0.25% of body weight offered once daily. Grass hay was fed at ∼1.5% to 2% of body weight, divided into similar a.m. and p.m. portions. Feed was withheld from respective horses on their scheduled day of necropsy. Potable water was available *ad libitum* and was supplied in two 16-L buckets per stall.Horses were housed under roof, but were otherwise exposed to ambient conditions for the duration of the study.

2.4. Outcome measures

2.4.1. Body weights

Individual body weights (kg) were measured on Day 1 to calculate accurate doses of MOX and FBZ. Each horse was weighed again just prior to euthanasia and necropsy. The livestock scale used for measuring body weights had been certified by a licensed scale service within three months before initiation of the study. Before and after each weighing session, the accuracy of the scale was verified with standard test weights ranging from 45 to 363 kg.

2.4.2. Fecal egg counts

Fecal samples were collected on Day 3 of the acclimation period, and numbers of strongylid eggs were counted in triplicate by the mini-FLOTAC procedure with a sensitivity of 5 eggs per gram (EPG) (Barda et al., 2014). Baseline egg counts were used to rank candidates within blocks for allocation.

Individual fecal samples were collected on Days 3, 11 and 18 (seven and 14 days after completion of larvicidal treatment). FECR on Days 11 and 18 was evaluated with an open-source Bayesian analysis tool (http://www.math.uzh.ch/as/index.php?id=eggcounts), and reported as mean FECRs with confidence intervals.

2.4.3. Necropsy and total worm count procedures

On Days 18–20, two replicates of horses were euthanatized daily and necropsied for nematode recovery. Euthanasia was conducted in compliance with current AVMA Guidelines (Leary et al., 2013). The root of the cranial mesenteric artery (CMA) was harvested from