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Original Article

Efficacy of two extra-label anthelmintic formulations against equine strongyles in Cuba

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ABSTRACT

Equine cyathostomin parasites are ubiquitous in grazing horses and have been shown to cause severe inflammatory disease in the large intestine of horses. Decades of intensive anthelmintic therapy have led to widespread anthelmintic resistance in cyathostomins across the world. In Cuba, no anthelmintic products are formulated and sold for equine usage and little is known about anthelmintic efficacy of ruminant and swine formulations used. A strongyle fecal egg count reduction test was used to assess the efficacy of a liquid formulation of ivermectin labelled for use in swine, ruminants and carnivores and a pelleted formulation of albendazole labelled for usage in ruminants. Nine farms in the province Camagüey were enrolled in the study comprising 149 horses in total. Albendazole efficacy was reduced on five farms and with the other four farms having no signs of reduced efficacy. Mean farm efficacies were ranging from 41.7% to 100% on the tested farms. Coprocultures found large strongyle larvae present on all farms, but all larvae identified post-treatment were cyathostomins. Ivermectin was found 100% efficacious on all studied farms. This study provided evidence of reduced albendazole efficacy in the study population. Further work is needed to evaluate whether these findings reflect true resistance or if they are due to pharmacokinetic or pharmacodynamic characteristics of the pelleted formulation tested here.

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1. Introduction

Equine cyathostomin parasites are ubiquitous in horses world-wide and generally recognized as important parasitic pathogens (Love et al., 1999). The clinical disease syndrome, termed larval cyathostominosis, is characterized by an acute generalized typhlocolitis with a profuse watery and protein-losing diarrhea and a reported case-fatality rate of 50% (Love et al., 1999).

Traditional parasite control programs rely on rotational anthelmintic treatments applied at regular intervals year round (Lloyd et al., 2000). This approach is based on concepts and strategies developed more than forty years ago (Nielsen, 2012), when *Strongylus vulgaris* (large strongyle bloodworms) was the major parasitic pathogen in horses (Drudge and Lyons, 1966). However, these interval-dose programs have led to wide-spread anthelmintic resistance in cyathostomin populations (Kaplan, 2002; Kaplan and Vidyashankar, 2012; Matthews, 2014; Peregrine et al., 2014). Additionally, new anthelmintic classes are unlikely to be approved for use in horses any time soon (Lyons and Tolliver, 2012). Accordingly, it is essential to assess the efficacy of the currently available products to make conscious decisions for treatment and control.

Benzimidazole resistance has been reported as ubiquitous in cyathostomin parasite populations in industrial countries across the world and resistance to pyrantel salts is found to be increasingly common as well (Lester et al., 2013; Lind et al., 2007; Traversa et al., 2012; Peregrine et al., 2014). Furthermore, strongyle egg re-appearance periods (ERP) following ivermectin and moxidectin treatment reportedly have decreased to about 4–5 weeks (Rossano et al., 2010; Lyons et al., 2011; Canever et al., 2013; Molento et al., 2008; von Samson-Himmelstjerna et al., 2007). Terminal studies have documented that these findings appear to be associated with emerging resistance in luminal L4 larvae (Lyons et al., 2009, 2010; Lyons and Tolliver, 2013).

Few equine parasitology surveys have been conducted in Cuba, and only two of these evaluated ivermectin treatment efficacy (Arece et al., 2002; Salas-Romero et al., 2014). As a result, very little is known about anthelmintic efficacy status of various available formulations. Thus, the aim of this study was to provide further information about the observed efficacy of frequently used formulations of ivermectin and albendazole against cyathostomin populations in Camagüey, Cuba.

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2. Materials and methods

Horses were allocated to the study between February and April 2014. The initial screening included 327 horses from 59 owners, who agreed to be part of the trial. The inclusion criteria were: equines older than six months, unpregnant mares, and strongyle fecal egg counts exceeding 500 eggs per gram (EPG). All horses had access to pasture and had not received any antiparasitic treatment in the last six months prior to the study.

Fecal egg counts from all horses were determined using a McMaster technique with a 25 EPG detection limit (Nielsen et al., 2013). Four grams of feces were put into a container with 26 ml of saturated sucrose salt (specific density of 1.21). The suspension was thoroughly homogenized and strained through a wire mesh to remove large debris. The strained suspension was collected in a beaker and thoroughly mixed. Then, 0.5 ml aliquots were added to each of two chambers of a McMaster slide. After 10 min, strongyle eggs under the two grids located within the chambers of the McMaster slides were counted under a light microscope at 100× magnification.

Fecal egg count reduction tests (FECRT) were performed with 149 horses originating from two state equine farms (Rancho San Vicente and the Equine Center from Triangulo 3 Agricultural Company); three small farms (Rancho Alegre, La Espezanza and Aranda), from the Cooperative José Antonio Echevarría; two groups from the Agricultural Fair of Camagüey (Equitación and Rodeo); and draft horses from individual owners in the areas of Nadales and Saratoga, in the city of Camagüey. Albendazole efficacy was evaluated on all nine farms, whereas four of the farms also participated in the ivermectin efficacy assessment.

2.1. Anthelmintic treatments

Body weights were estimated by weight tape (Stratford et al., 2014), and each horse was administered a dose calculated for 110% of its estimated weight. Because anthelmintics approved for equids are relatively unavailable in Cuba, two products that are frequently used extra-label for horses in Camaguey were tested. Micronized albendazole (7.5 mg/kg) originated as Albendazol® (Labiofam Cuba, Havana, 112 Cuba), 700 mg tablets, and was administered orally. Similarly, ivermectin (0.2 mg) was tested as Labiomec® (Labiofam Cuba, Havana, Cuba), a 1% solution for parenteral use in pigs, carnivores and ruminants, but administered orally to horses in the present study.

2.2. Coproculture

Fecal samples collected from each farm on days 0 and 14 were pooled and processed for coproculture. A minimum of 3 g from each strongyle-positive sample were mixed together and incubated for 14 days at room temperature in the lab (24–29 °C). Approximately 300 third-stage larvae (L3) were collected using the Baermann apparatus technique, and identified according to published morphological criteria (Russell, 1948).

2.3. Data analysis

Efficacy was determined by the Fecal Egg Count Reduction Test (FECRT), based on the difference between the arithmetic means of pre and post treatment EPGs, using Microsoft Office Excel® (2007), and the formula below:

$$\text{FECR} = \left(\frac{\text{EPG}_{\text{pre}} - \text{EPG}_{\text{post}}}{\text{EPG}_{\text{post}}}\right) \times 100\%$$

Data were then analyzed using SAS, version 9.3 (Cary, NC, USA). The logistic procedure was used for analysis of differences between farms and the possible influence of pre-treatment egg count level on the FECR results. Results were interpreted at the 0.05 level.

2.4. Interpretation of results

The FECRT results were classified according to threshold values recommended by Matthews (2014) and Nielsen et al. (2013), for IVM, and the thresholds for ALB were adapted from those recommended for BZs. The thresholds used are presented in Table 1.

3. Results

Egg counts revealed that horses were parasitized by strongyles and *Parascaris* spp. Eggs of *Oxyuris equi* were identified in three horses. No eggs from cestoda or trematoda were identified. Pre-treatment coprocultures identified large strongyle and cyathostomin larvae in all investigated farms (Table 2).

3.1. Albendazole efficacy

Overall, ALB efficacy was assessed in 113 horses from 9 groups. Reduced ALB efficacy was observed in five groups (Table 3), and the remaining four farms had no signs of reduced efficacy according to the criteria used. All larvae observed post treatment were identified as cyathostomins. The logistic regressions revealed significant differences between farms ($p < 0.0001$), and the resultant Odds Ratios are presented in Table 3. The efficacies of San Vincente and Triangulo Tres were significantly higher than those observed on the other seven farms but were not statistically significant from each other. Pre-treatment strongyle egg count levels were shown to be statistically associated with the fecal egg count reduction calculation ($p = 0.0164$).

3.2. Ivermectin efficacy

The efficacy of IVM was tested on four farms, with a total of 35 individuals (Table 4). Efficacy was 100% in all four cases. (See Table 4.)

4. Discussion

This is the first report of reduced anthelmintic efficacy against equine cyathostomins in Cuba. Both of the anthelmintic formulations evaluated in this study were formulated for animal species other than horses, and using them in horses can thus be considered extra-label usage. There are no anthelmintic formulations produced or sold for equine usage in Cuba. Therefore, extra-label use of ruminant or swine formulations has been the only alternative for many years. For the past 15 years, horses in Camaguey have been treated almost exclusively with the two formulations evaluated here.

Although the albendazole efficacy data reported here suggest anthelmintic resistance, it cannot be unequivocally interpreted as such as the no baseline data exist for the formulation tested. However, resistance seems to be a genuine possibility in the Camaguey province given previous reports of improper use of anthelmintic formulations with erroneous weight estimations and ignorance regarding dosage and route of administration (Guerra et al., 2005). Furthermore in Cuba, there are practically no restrictions to purchasing veterinary medication, although resultant anthelmintic treatment intensities are often as low as one treatment per horse per year.

Table 1
Efficacy thresholds used for classifying reduced anthelmintic efficacy, suspected reduced anthelmintic efficacy, or no signs of reduced anthelmintic efficacy for each of the two anthelmintics evaluated.

Anthelmintic	Reduced efficacy	Suspected reduced efficacy	No signs of reduced efficacy
Ivermectin	<95%	95–98%	>98%
Albendazole	<90%	90–95%	>95%