Veterinary Parasitology: Regional Studies and Reports 13 (2018) 220–223

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr

Original Article

First report of anthelmintic resistance of equine cyathostomins in Cuba

J. Salas-Romero[a,*], K.A. Gómez-Cabrera[a], J.E. Salas[b], R. Vázquez[a], A. Arenal[a], M.K. Nielsen[c]

[a] Facultad de Ciencias Agropecuarias, Universidad de Camagüey Ignacio Agramonte Loynáz, Cuba
[b] Escuela Fiscal Unidad Educativa Nacional Napo, Sucumbios, Ecuador
[c] M.H. Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY, USA

ARTICLE INFO

Keywords:
Cyathostomins
Equines
Fenbendazole
Oxibendazole
Anthelmintic resistance

ABSTRACT

Anthelmintic resistance in equine cyathostomins has been described worldwide, with resistance to the benzimidazole class being particularly widespread. The status of anthelmintic efficacy in Cuba has been virtually unknown due to the lack of equine labelled products. One recent report documented suboptimal efficacy levels of extra-label albendazole products against cyathostomins, but it remains unknown to which extent benzimidazole resistance exists in the population. The aim of the present study was to evaluate the anthelmintic efficacy of two benzimidazole products labelled for equines, fenbendazole and oxibendazole. A fecal egg count reduction test (FECRT) was carried out on 132 horses aged 4 months to 18 years in 14 herds, belonging to six provinces. Ten herds exhibited signs of resistance to at least one of the benzimidazoles (mean FECRT < 90%). Overall, oxibendazole exhibited higher efficacy than fenbendazole ($p = 0.0062$), and higher efficacy levels were found in horses never dewormed before compared to those treated within 3–12 months prior to the study ($p = 0.0015$). Pre-treatment larval cultures revealed the presence of large strongyles and cyathostomin larvae in all herds, while only cyathostomin larvae were detected post treatment. The present work is the first report of anthelmintic resistance in equine cyathostomins in Cuba, and suggests pre-selection for resistant strains by extra-label use of albendazole on the studied farms.

1. Introduction

Equine internal parasites are ubiquitous in horses across the world. Cyathostomins are the most prevalent and abundant parasites of equines for almost all ages, regardless of the time of year (Kuzmina et al., 2016). These parasites can cause larval cyathostominosis which is an acute typhlo-colitis characterized by diarrhea, dehydration, and protein loss (Love et al., 1999). The case fatality rate of this condition has been reported to be around 50% (Reid et al., 1995).

Cyathostomin parasites have been controlled through the use of various anthelmintic compounds formulated for equine usage. In Cuba, however, such equine products are generally not available, and horse owners have been forced to use bovine or porcine products on an extra-label basis (Salas-Romero et al., 2017a). Worldwide, anthelmintic resistance is widely reported in cyathostomin parasites, with benzimidazole and pyrantel resistance being highly prevalent, and macrocyclic lactone resistance emerging (Peregrine et al., 2014). Due to the near exclusive use of extra-label anthelmintic products in Cuba, concerns have been raised about the current status of anthelmintic efficacy in this country. A recent study evaluated the efficacy of commonly used extra-label products, and found reduced efficacy of two different formulations of albendazole, while oral administration of an injectable formulation of ivermectin was generally effective (Salas-Romero et al., 2017a). Although these findings suggest benzimidazole resistance in Cuban cyathostomin parasites, an affirmative diagnosis can only be achieved by evaluating the efficacy of anthelmintic products developed and approved for use in equines.

The aim of the present work was to evaluate the efficacy of two benzimidazole anthelmintic products labelled for equine usage against Cuban strongylid parasites.

2. Materials and methods

The study included 132 horses from 14 herds. Horses were allocated to the study between September 2016 and March 2017, and included six Cuban provinces (Artemisa, Villa Clara, Sancti Spíritus, Ciego de Ávila, Camagüey y Holguín). The location of the farms can be viewed in Fig. 1. The inclusion criteria were as follows: equines older than six months, unpregnant mares, previous extra-label use of albendazole on farm, and pretreatment strongyle fecal egg counts exceeding 625 eggs per gram (EPG). All horses had access to pasture and had not received any antiparasitic treatment for three months prior to the study. Table 1

* Corresponding author.
E-mail address: josmel.salas@reduc.edu.cu (J. Salas-Romero).

https://doi.org/10.1016/j.vprsr.2018.07.005
Received 11 October 2017; Received in revised form 22 May 2018; Accepted 11 July 2018
Available online 17 July 2018

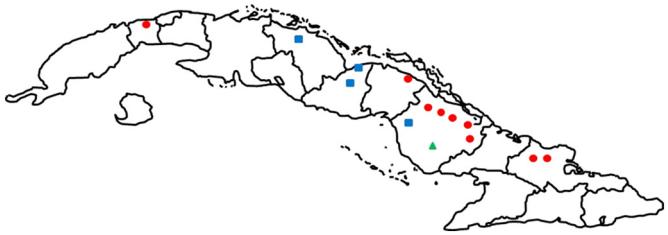

Fig. 1. Map with the location of the 14 participating farms in the study. Benzimidazole resistance was identified on nine farms (circles) and one farm had resistance to fenbendazole, but not oxibendazole (triangle). Four farms had no signs of benzimidazole resistance (squares).

presents a summary of the study population.

2.1. Anthelmintic treatments

Body weights were estimated by equal proposed by García Neder et al. (2009), whereas in the case of Rancho Azucarero horses it was determined with scales. Each horse was administered a dose calculated for 110% of its estimated weight as described in the literature (Stratford et al., 2014).

The efficacies of fenbendazole (7.5 mg/kg, Meltra® oral, Brouwer S.A. Argentina) and oxibendazole (10 mg/kg, Anthelcide® EQ, Zoetis Inc. USA) were estimated, if the number of horses present per herd to reach a minimal of horses per both group.

2.2. Fecal egg count

Fecal egg counts 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.

Approximately 20 g of feces were collected directly from the rectum, or from e freshly excreted fecal pile. The fecal material was labelled and transported at 4 °C to the laboratory. Strongyle fecal egg counts were determined at the day of treatment (Day 0) and at two weeks post treatment (Day 14).

The samples were processed in the Laboratory of Parasitology of the University of Camagüey "Ignacio Agramonte Loynaz", except the samples of the Rancho Azucarero, which were analyzed at the National Laboratory of Parasitology, in Artemisa province. All samples were processed within 72 h after collection.

2.3. Coproculture

Coprocultures were carried out with the feces of the horses representing each population on Day 0 in order to characterize the strongylid fauna present in each group. Coprocultures were also carried out on Day 14 in those herds where resistance to one of the anthelmintics was diagnosed. A minimum of 3 g from each strongyle-positive sample were mixed together and incubated in glass beakers for 14 days at room temperature in the laboratory (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; Bevilaqua et al., 1993).

2.4. 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 (Nielsen et al., 2013):

$$FECR(\%) = \frac{(EPGpre - EPGpost)}{EPGpost} x\ 100\%$$

Statistical analyses were carried out using SAS, version 9.4 (SAS Institute, Cary, North Carolina, USA). A multivariate mixed linear analysis was carried out with log-transformed strongyle fecal egg count reduction (FECR) as the response variable and age, sex, anthelmintic, and time since last deworming as covariates and equine population province as random effect. The model was constructed using forward addition and backward elimination of covariates. All covariates with a p-value of 0.20 or below were kept in the model.

2.5. Classification of resistance

This study followed guidelines described by the American Association for Equine Practitioners for classifying anthelmintic resistance in equine cyathostomins (Nielsen et al., 2013). For fenbendazole and oxibendazole, mean FECRs above 95% are classified as no sign of resistance, the 90–95% is considered suspect resistance, and below 90% is clear evidence of anthelmintic resistance.

3. Results

Table 2 summarizes the FECRT data generated in the study. Ten of the 14 studied herds had resistance to at least one of the benzimidazoles evaluated. Overall, oxibendazole efficacy levels were significantly higher than for fenbendazole ($p = 0.0062$), and horses never dewormed before exhibited significantly higher treatment efficacies than horses dewormed within 3–12 months prior to the study ($p = 0.0015$). Finally, pre-treatment strongyle FEC was significantly positively associated with anthelmintic efficacy ($p = 0.0021$).

Table 3 presents the large strongyle L3 s that were identified pretreatment, while cyathostomins were the most abundant. However, in resistant herds, only larvae of cyathostomas were observed in the coprocultures of day 14.

4. Discussion

The present work constitutes the first report of anthelmintic resistance in horses of Cuba as this is the first time equine anthelmintic products have been evaluated. These herds had all been previously treated with extra-label benzimidazole products (Table 1), which likely selected for resistant strains of cyathostomin parasites. It is worth mentioning that four of the herds evaluated in this study were previously part of a study evaluating the efficacy of an extra-label albendazole product (Salas-Romero et al., 2017a) and efficacy levels were comparable to those observed for oxibendazole in the present study.

The high prevalence of resistance to benzimidazoles, detected throughout the island in this study is deeply concerning. Worldwide, benzimidazole resistance in equine cyathostomins is the most prevalent and is reported in at least 14 countries (Peregrine et al., 2014). In countries like the United States, > 95% of the herds have been reported resistant to at least one benzimidazole (Kaplan et al., 2004). An evaluation of fenbendazole efficacy on a total of 80 yards of the United Kingdom, German and Italy yards revealed resistance present on > 80% in the UK and Germany and on 38% of Italian yards (Traversa et al., 2009). Thus, Cuban findings reported here are in agreement with world-wide trends. Furthermore, the difference in efficacy observed between the two benzimidazoles evaluated is in agreement with international studies documenting that oxibendazole