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Equine Cyathostomin Resistance to Fenbendazole in Texas Horse Facilities

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A B S T R A C T

Cyathostome resistance to the benzimidazole fenbendazole (FBZ) and other anthelmintic medication has been documented worldwide. Parasite resistance to anthelmintic medication is of great concern to the anthelmintic industry and to horse owners. The present study examines the efficacy of FBZ anthelmintic treatments in horse herds from ranches in four different geographical locations within Texas. In addition, the load reduction method was compared with the traditional fecal egg count reduction test to determine the incidence of parasite resistance to FBZ. Four ranches in different areas of Texas were surveyed to determine cyathostome resistance to FBZ. Two of the four ranches had young (aged ≤2 years) and older animals (aged >2 years). The number of animals with parasites varied widely between the study's locations. Differences were observed in both the fecal egg count reduction test and load reduction method across the four study sites ($P < .001$). Cyathostome resistance to FBZ seemed to be prevalent in three of the four ranches, whereas FBZ was highly efficacious against cyathostomes on one ranche. There was a trend toward increased parasite resistance in the younger animals ($P = .081$). These results show the importance of testing anthelmintic medication effectiveness.

1. Introduction

The prevalence of anthelmintic-resistant intestinal parasites is a rapidly growing problem in the equine industry. Parasites can cause extensive and irreparable damage to the animal's internal anatomy and disrupt the normal physiology. In addition, parasites jeopardize performance, well-being, and health, and can cause mortality in horses [1-5].

Currently, of all the parasites, cyathostomins have emerged as a major equine problem and are the most widespread. Cyathostomin pathogenicity varies on an individual basis. Clinical signs may include weight loss, poor feed efficiency, dull hair coat, diarrhea, and intermittent colic, among other symptoms [2].

Resistance to an anthelmintic treatment is a heritable ability of an individual within a parasite population to tolerate doses of an anthelmintic compound more effectively than healthy individuals within a treated group, where previous efficacy was demonstrated [3]. Parasite resistance to anthelmintic medications has been seen worldwide, including the United States, and has been a subject of numerous reviews [4-12]. Equine parasite species have been shown to be resistant to all classes of anthelmintic compounds [12], including macrocyclic lactones [9,10,13].

Cyathostomins have widespread resistance to benzimidazoles, and studies have suggested widespread prevalence of resistance, especially to fenbendazole (FBZ) [14,15]. This may be because of several factors, including the fact that benzimidazoles are one of the oldest classes of anthelmintic medications and are still widely used in

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horses. In addition, other factors include overuse of benzimidazoles without rotating between classes of anthelmintic medication, underdosing animals, genetic resistance, lack of knowledge of the biology of cyathostomins, and cyathostomin encystment in the gut wall of the host [16-18]. Of these possible factors, cyathostomin encystment in the intestinal wall warrants increased concern, as most anthelmintic medications are not labeled to control encysted larvae. Encystment periods may range from 4 months to as long as 2 years [17] and may be influenced by time of year (lack of nutrients in the winter, heat stress during summer months), physiological cues attained from the host (chemofactors during stress), the amount of nutrition density, and the diet of the host [19].

Texas is a diverse state, and management of horse ranches is not similar to many equine facilities found in the East/Southwest, where the majority of U.S. parasite resistance studies have been focused. Climatic conditions are variable across this large state, and further, management varies greatly across ranches in Texas in response to terrain, rainfall, size of ranch, stocking rates, and use of horses. The present study examines the efficacy of FBZ anthelmintic treatments in horse herds from ranches in four different geographical locations within the state. The further objective of this study was to develop a load reduction (LR) categorization method of analysis to assess parasite resistance.

2. Materials and Methods

The present study included 169 horses from four different ranches representing four different geographical locations in Texas. These horses had not been exposed to anthelmintic treatment for >2 months before the initiation of the study. Timing of the treatment with FBZ varied throughout the year (Table 1).

Pretreatment fecal samples were collected from a fresh pile of feces from an identified individual animal using an inverted sealable bag, or if stocks were provided, samples were collected using a rectal fecal grab method. All air was expelled from the sealable plastic bags. The samples were then placed in a cooler with ice until they could be transported to the Equine Parasite Evaluation Lab at Texas Tech University to be analyzed within 24-48 hours. Once the fecal samples were collected, the weight of each horse on ranches 2-4 was determined by using an equine weight tape (Intervet Inc., Millsboro, DE), and the weight of each animal was rounded up to reduce the risk of underdosing. On ranch 1, a certified livestock scale was available, and each horse was individually weighed. Each horse was then treated with the correct dose, as determined by its weight, with 5 mg/kg body weight of Safe-Guard (Merck Animal Health, DeSoto, KS), administered intraorally to each horse. Fourteen days after the anthelmintic treatment and initial sample collection, a posttreatment fecal sample was taken from each horse. The parasite evaluation for the posttreatment was conducted using the same procedures as used for the pretreatment.

2.1. Fecal Analysis

The modified Wisconsin sugar flotation method [19-22] was used to analyze fecal egg count (FEC). For this method, the detection limit of the egg counting technique is 0.3 egg/1 g of fecal sample [20]. Fecal samples were massaged and mixed in the bag, and 3 g of feces was measured and mixed with 15 mL of hyperosmotic sugar solution (specific density: 1.27). Samples were strained through a small mesh metal strainer into a 15-mL test tube and were spun in a swinging arm centrifuge (Garver Electrifuge, Model 008, Union City, IN) at approximately 1000 rpm for 7 minutes. Coverslips were placed on the test tubes for 5 minutes and subsequently placed on slides for microscopic quantification [21].

2.2. Fecal Egg Count Reduction Analysis

Sampling of fecal material posttreatment was conducted 14 days after the initial sampling and treatment following the same procedures. Fecal egg count reduction analysis was determined using the following calculation, $100 - ([\text{posttreatment}/\text{pretreatment}] \times 100)$. For the FECR analysis, resistance was defined at the 90% threshold.

2.3. LR Analysis

In addition to FECR analysis, a LR method was used to categorize the FECR analysis results (Table 2). The LR categories ranged from 1 through 3. A designated LR1 was assigned to FECR of values ≥90%, LR2 to FECR analysis of 1-89%, and LR3 to FECR analysis of ≤0%. LR1 indicated a true treatment effect; LR2 indicated a subclinical effect; and LR3 indicated ineffectiveness.

2.4. Statistical Analysis

The statistical analysis was conducted by using raw data of pre- and posttreatment FECs to determine FECR. They were first analyzed independently by age and ranch using analysis of variance. Where appropriate, mean separation was then performed using Tukey mean separation. Data were also analyzed in a multifactorial design by ranch and age. As described previously, the formula for FECR created large variances where treatments were ineffective

Table 1
Treatment with 5 mg/kg body weight of FBZ (Safeguard)

	November 2008	March 2009	June 2009	August 2009
Ranch 1	FBZ			
Ranch 2		FBZ		
Ranch 3			FBZ	
Ranch 4				FBZ

FBZ, fenbendazole.

Table 2
LR categories: LR1 indicates FECR analysis of ≥90% egg reduction, LR2 indicates FECR analysis of 1-89% egg reduction, and LR3 indicates FECR analysis of 0% egg reduction

LR categories	FECR (%)
LR1	≥90
LR2	1-89
LR3	0

LR, load reduction.