Parasite Resistance and the Effects of Rotational Deworming Regimens in Horses

Heidi A. Brady, PhD, Diplomate ACAP; Wade T. Nichols, PhD*; Megan Blanek, MS; and David P. Hutcheson, PhD

Three studies examined parasite resistance and efficacy of anthelmintic rotational regimens in the horse. These studies showed that a rotational regimen could effectively reduce or overcome the resistance of parasites to fenbendazole and that fenbendazole could be effectively used in subsequent rotational schemes. Authors' addresses: Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX 79409 (Brady, Blanek); Intervet, Inc., Millsboro, DE 19966 (Nichols); and Animal-Agricultural Consulting, Inc., Amarillo, TX 79159 (Hutcheson); e-mail: heidi.brady@ttu.edu.
*Presenting author.

1. Introduction

The misuse or overuse of one anthelmintic has been implicated in the possible spread of parasite resistance to that particular anthelmintic. This three-part study examined the effects of fenbendazole (FBZ) on parasite resistance and possible means to overcome or reduce this resistance.

Parasite resistance has emerged as a dilemma in the livestock industry because many parasites are becoming resistant to all classes of anthelmintics. The mechanisms of parasite resistance to current equine anthelmintics have been reviewed.[1] New problems concerning resistance in small strongyles (cyathostomes) to benzimidazole (BZM) have been documented worldwide.[2–4] Various rotational schemes between classes have been suggested, including slow- and fast-rotation plans, with the goal of the prevention of parasite resistance; however, few studies have examined these plans.

Resistance has been reported in every main class of anthelmintics in the horse. Unfortunately, there are many definitions for resistance in the scientific literature. Studies have reported on cyathostome resistance to BZM since the 1960s.[5,6] Many reports of resistance to this class have been associated with the frequent administration of this product to a herd for a significant period without rotation between different classes of anthelmintics or with frequent dosing intervals.[7]

This research consisted of a three-part study in which anthelmintic treatment dosage and the rotational regimens of different chemical classes were evaluated in a herd of horses administered FBZ for 18 mo before evaluation of resistance and efficacy. If a resistance could be established through fecal egg-count reduction (FECR) tests and other anthelmintics were found to be efficacious, then the hypothesis developed was that a rotation among different classes of anthelmintics may reduce or overcome the resistant parasites and allow for the use of FBZ again on this farm.

2. Materials and Methods

For the purpose of investigating possible anthelmintic resistance in horses, a herd of Quarter Horses, housed at the Texas Tech Ranch Horse Center in New Deal, Texas, were administered only FBZ

NOTES

every 90 days for 18 mo without rotation with any other class of anthelmintic.

Experiment 1

In a September 2003 study, the 28 Quarter Horses used were 13 mares, 3 yearling fillies, 8 weanling fillies, and 4 weanling colts. All horses were housed at the Texas Tech University Ranch Horse Center. Horses were stratified by age, sex, fecal egg counts (FEC; taken on day 3), and pregnancy status. On day 0, all horses were treated with one of three different doses of FBZ. Group 1 was treated with 5 mg/kg body weight (BW) of FBZ[a] on days 0, 28, and 56. Horses in group 2 were treated with 10 mg/kg BW of FBZ on the same days. Horses in group 3 were treated with 10 mg/kg BW[b] daily for 5 consecutive days (50 mg/kg BW) on days 0, 28, and 56.

In addition to the baseline sample (day 3), a fecal sample was collected from each horse on days 0, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 98, 112, 126, and 140. All fecal samples were collected rectally while animals were restrained in stocks and were sent to Animal Production Consulting in Lincoln, Nebraska.[c] FECs were determined by the Wisconsin Sugar Flotation Method. Results were reported as worm eggs in 1 g of manure, and parasite eggs were identified as ascarids or strongyles.

On day 0, blood was taken by jugular venipuncture for tapeworm diagnostic testing. Blood samples were allowed to clot for 1 h and were then centrifuged for 10 min. One milliliter of serum was transferred to another labeled tube and sent for analysis for the presence of tapeworms. An enzyme-linked immunosorbent assay (ELISA)[d] was used to measure antibodies against antigens to *Anoplocephala perfoliata*.

A composite fecal sample was taken from the weanlings and yearlings on day 0 to identify and compare resistance to the different anthelmintics. The DrenchRite®[e] LDA in vitro assay was used to detect the larval resistance to BZM, levamisole, BZM/levamisole combination, and avermectin/milbemycin anthelimintics; a microtiter plate larval plate assay was used.[8] All fecal samples were sent to Texas A&M College of Veterinary Medicine in College Station, Texas.[f] The lab technicians were blinded as to treatment and samples.

Statistical Analysis for Experiment 1

A randomized complete block design was used. Horses were blocked by age (young and mature). The treatments were 5 mg/kg BW FBZ, 10 mg/kg BW FBZ, and 50 mg/kg BW FBZ. The data were analyzed by analysis of variance (ANOVA). Means were separated by least significant difference.[g] FECR analysis was determined 14 days after each treatment by using the following equation:

$$\text{FECR} = 100 - [(\text{post-treatment worm count}/\text{pre-treatment worm count}) \times 100]$$

Experiment 2

Three months after the last treatment on experiment 1 (day 154), the same horses were randomized by age, FEC taken on day 140 of experiment 1, pregnancy status, and previous treatment group. They were then assigned to one of three treatment groups.

Group 1 was treated with 0.4 mg/kg BW of moxidectin,[h] group 2 was treated with 200 mcg/kg BW of ivermectin,[i] and group 3 was treated with 50 mg/kg BW FBZ over 5 consecutive days.[b] After fecal collection on day 0, dosages were determined by the horses' individual weight as recorded on day 140.

Fecal samples were collected on days 154, 161, and 168 and sent to Animal Production Consulting. The lab technicians were again blinded as to treatment and samples. Quantative egg counts were determined by the Wisconsin Sugar Flotation Method. Results were reported as parasite eggs per one gram of manure, and parasite eggs were identified as ascarids or strongyles.

Statistical Analysis for Experiment 2

A randomized complete block design was used. Horses were blocked by age (young and mature). The treatments were moxidectin, ivermectin, or 50 mg/kg BW FBZ. The data was analyzed by ANOVA. Means were separated by least significant difference. χ^2 tests were used to evaluate resistant versus non-resistant status at a 90% reduction level. FECR analysis was determined 14 days after the treatment by the following equation:

$$\text{FECR} = 100 - [(\text{post-treatment worm count}/\text{pre-treatment worm count}) \times 100]$$

Experiment 3

Three months after the treatment of experiment 2, a 1-yr practical rotational program was implemented for every horse mean n = 26.6 housed at the Texas Tech Ranch Horse Center. This included many of the animals used in experiments one and two as well as stallions, foals, and outside mares. A quarterly rotation was used by treating all the horses with 1.36 mg/kg BW of pyrantel pamoate[j] in June (n = 31), 200 mcg/kg BW of ivermectin and 1 mg/kg BW of praziquantel[k] in September (n = 23), 50 mg/kg BW FBZ over 5 consecutive days in December (n = 21), and 0.4 mg/kg BW of moxidectin in March (n = 26). AQ: 1

Fecal samples were collected on the day of treatment for each anthelmintic and 7 days after the treatment. Quantitative egg counts were determined as detailed in experiments one and two.

Statistical Analysis for Experiment 3

The entire farm was used in experiment 3, and each horse was used as the experimental unit at each sampling period. Reduction analysis was determined 7 days after the treatment by the following equation:

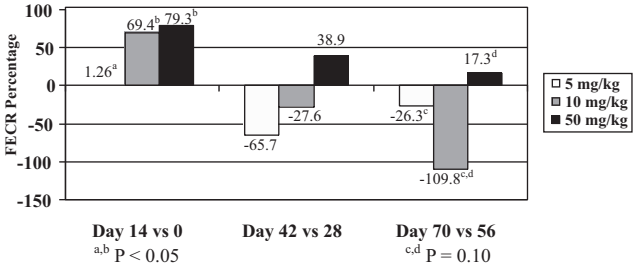

Fig. 1. FECR in horses administered 5, 10, or 50 mg/kg BW of FBZ over 5 days (larvicidal treatment).

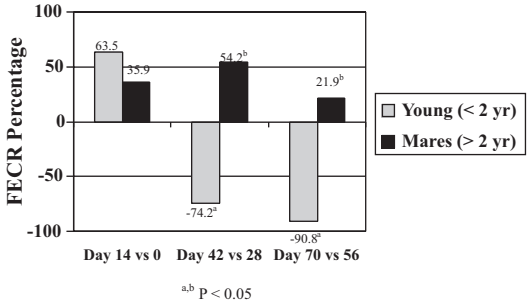

Fig. 2. FECR by age with all treatments combined in experiment 1 that compared young horses (≤2 yr of age) with mature mares (>2 yr of age).

$$\text{FECR} = 100 - [(\text{post-treatment worm count}/\text{pre-treatment worm count}) \times 100]$$

The FECR exhibited by each individual animal was analyzed by χ^2 analysis. χ^2 was used to evaluate resistance versus non-resistance status at both a 90% and 98% reduction level.

3. Results and Discussion

Experiment 1

The DrenchRite® assay, based on the initial composite fecal sample, showed mid-level cyathostome resistance to the BZM class. Testing for antibodies against antigens to *Anoplocephala perfoliata* with the ELISA assay proved negative for all horses. All ascarids were eliminated in each treatment by day 7 when analyzed by egg-differential analysis; therefore, all study analysis was based on cyathostome egg count.

The analysis of the three treatment groups by FECR 14 days post-treatment indicated resistance to FBZ (Fig. 1). In all groups and at all time points measured, the FECR was lower than the two thresholds (95% and 90%) used to measure efficacy of anthelmintics. Although there is not agreement in the literature over the definition of resistance, the World Association for the Advancement of Veterinary Parasitology defines resistance as FECR using arithmetic means <95%.[9] An alternate method has defined resistance as post-treatment FECR of <90%.[9] On day 14 versus day 0, the FECR for the 5 mg/kg BW group was significantly less than both the 10 mg/kg BW and the larvicidal 50 mg/kg group. There was no significant difference between groups on day 42 versus day 28. However, there was an increase in FEC after treatment in the 5 mg/kg BW and the 10 mg/kg BW groups. On day 70 versus day 56, there tended to be a difference between the 5 mg/kg and larvicidal groups. Furthermore, there was an increase in FEC 14 days post-treatment for the 5 mg/kg and the 10 mg/kg groups, which indicates resistance to this class. Large variability in FECR between the three groups was observed (illustrated in Fig. 1). On day 70 versus day 56, the 10 mg/kg dose was not statistically different from the larvicidal dose because of the large standard error (71.0) despite a large numerical difference in means.

All treatments were combined and examined by age of young horses (≤2 yr of age) and mature mares (>2 yr of age). There was a significant difference between groups on both day 42 and day 70 (Fig. 2). Both day 42 versus day 28 and day 70 versus day 56 had an increase in FEC in the young horses, which indicates resistance. Based on this data, it seems that the population of cyathostomes was more resistant to FBZ in the young horse group. However, it is important to note that this group had an overall higher pre-treatment FEC.

Mean eggs per 1 g of manure over time (days 0–140) are illustrated in Figure 3. The 50 mg/kg BW group was lower ($p < 0.05$) than both the 5 mg/kg BW and 10 mg/kg BW groups on days 7, 70, 77, 84, and 98. In addition, the 50 mg/kg group was significantly lower than the 5 mg/kg BW group on

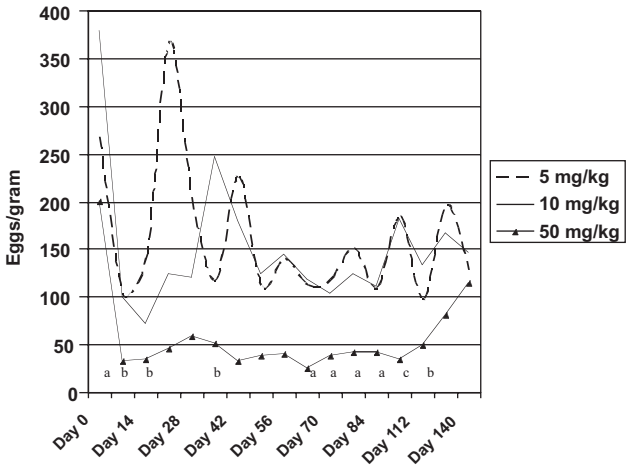

Fig. 3. Mean eggs per gram of feces over time (sampled every 7 days for 140 days) in horses administered 5, 10, or 50 mg/kg FBZ. Treatment days were days 0, 28, and 56. [a]50 mg/kg BW group was lower ($p < 0.05$) than both 5 mg/kg BW and 10 mg/kg BW groups. [b]50 mg/kg BW group was lower ($p < 0.05$) than the 5 mg/kg BW group. [c]50 mg/kg BW group was lower ($p < 0.05$) than the 10 mg/kg BW group.

days 14, 21, 42, and 126. Similarly, the 50 mg/kg BW group was significantly lower than the 10 mg/kg BW group on day 112. This indicates that very large doses may alleviate some resistance problems but will not totally eliminate the problem.

In experiment 1, resistance was documented in the herd after the horses were administered FBZ for 18 mo before the study without rotation. This was expected and is in agreement with previous studies in the literature showing parasite resistance to FBZ after prolonged use without rotation.[5,7,10–13]

Contributing factors to the development of resistance can include the high frequency of deworming, the continued use of only one class of anthelmintic, and the underdosing of the anthelmintic, either by underestimation of the animal's weight or by losing product during the administration. Studies in sheep have shown that the frequent use of an anthelmintic will directly affect the rate of selection of resistance.[14]

After day 7, parasitic egg burdens in this study were cyathostomes. This is also in agreement with the literature specifically documenting cyathostome resistance to FBZ and other BZMs.[5] In the horse, cyathostomes are the most difficult to treat because of the fact that they become encysted in the gut wall and are associated with general resistance. In this experiment, neither increased frequency (every 28 days) nor higher dosages (10 mg/kg BW or 50 mg/kg BW over 5 consecutive days) effectively decreased FEC to >90% of reduction levels in these horses. These findings are similar to those of Hutchens and DiPietro,[15] who found that biweekly treatment of 5 mg/kg BW of FBZ for 62 days on a BZM-resistant population of small strongyles in Standardbreds in Illinois was not effective. An additive effect was not present, which was determined by FECR, larval cultures, and egg-hatchability assays.

Experiment 2

Three months after the last treatment in experiment 1, three different anthelmintic regimens were examined to determine efficacy after resistance had been documented in the herd. Mean FECR percentages tested on day 14 post-treatment were 99.9% for the group treated with 0.4 mg/kg BW of moxidectin, 98.7% for the group treated with 200 mcg/kg BW of ivermectin, and 84.3% for the group treated with 50 mg/kg BW (larvicidal dose) of FBZ (Fig. 4). Both the moxidectin and ivermectin groups showed significantly higher FECR than the group treated with FBZ.

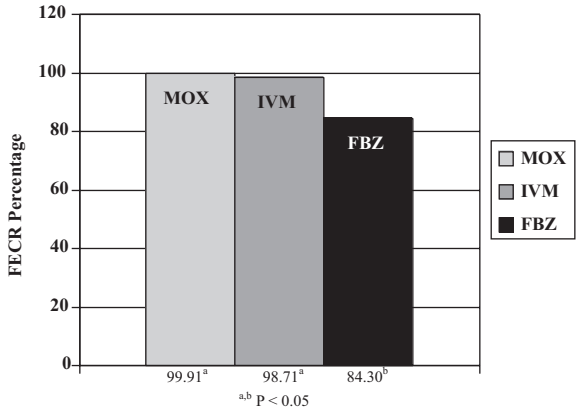

Fig. 4. FECR in horses administered moxidectin (n = 11), ivermectin (n = 11), and FBZ (n = 11).

The mean FECR of the young horses versus mature mares is illustrated in Table 1. The young horses treated with moxidectin had a 99.8% reduction, and the mares had a 100% reduction. The young horses treated with ivermectin had a reduction of 97.6%, and the mature mares had a 100% reduction. However, the young horses treated with FBZ had only a 69.9% reduction, whereas the mares had a reduction of 98.7%. The young horses treated with FBZ were significantly different than the mares treated with FBZ as well as the groups treated with ivermectin and moxidectin. FECR in mares treated with FBZ was not different ($p > 0.05$) from the mares treated with ivermectin and moxidectin.

χ^2 analysis was used to evaluate resistance versus non-resistance at a 90% reduction level. When all groups were combined, the moxidectin and ivermectin groups were similar ($p > 0.05$), and both were significantly different from the FBZ group ($p < 0.05$).

The treatments of moxidectin and ivermectin both proved to be effective, because their reduction levels were all >98%. However, FBZ proved to be less effective, because the reduction of FECs were below 90%, which does not meet the level set by the Food and Drug Administration (FDA).

As in experiment 1, the age effect was clear. There were no differences between treatment groups (moxidectin, ivermectin, and FBZ) in the mature mares. However, there was a large difference in cyathostome egg per one gram of manure between the young horses treated with FBZ and the mature mares treated with FBZ. This indicates a greater problem of cyathostome resistance to FBZ in the young horse. This may be caused by several factors including decreased immune response in the young horses as well as overall higher egg counts.

Experiment 3

Three months after the end of experiment 2, a quarterly anthelmintic rotational program was implemented on the farm. Mean FECR percentages on day 7 of post-treatment were 95.86% with pyrantel pamoate, 100% with ivomectin and praziquantel, 97.84% with FBZ (larvicidal dose), and 100% with moxidectin (Table 2). χ^2 analysis was used to evaluate resistant versus non-resistant status at both a 90% and 98% reduction level. Mean FECR was not different from both a 90% and 98% reduction at all treatment times.

In experiment 3, a quarterly rotational scheme (fast rotation) was used to evaluate parasite control

Table 1. FECR Young (≤2 yr) and Mature (>2 yr) Horses Administered MOX, IVM, and FBZ

Treatment	Age	FECR	SEM
Group 1 (MOX)	Young	99.8*	0.17
	Mature	100*	0
Group 2 (IVM)	Young	97.6*	2.30
	Mature	100*	0
Group 3 (FBZ)	Young	69.9†	15.8
	Mature	98.7*	0.79

*†$p < 0.05$.

in a herd that had been proven to be resistant to FBZ. Some researchers have suggested that after resistance has been established to an anthelmintic, it is not advisable to go back to that class.[7,16] In contrast, this study showed that resistance to FBZ in a herd can be broken or reduced by using a fast (quarterly) rotation of different classes of anthelmintics. Additionally, FBZ at the larvicidal dose can be effectively implemented in a rotational scheme despite prior resistance.

Previous literature does not agree on the use of rotational schemes to decrease resistance. Several rotational plans have been suggested for the efficient control of parasites in the horse. Programs including interval dosing, targeted dosing, strategic dosing based on egg reappearance period (ERP), and continuous in-feed dosing have been reviewed.[17] There is also disagreement between the efficacies of slow- versus fast-rotation plans. Fast or rapid rotational schemes, such as the plan used in experiment 3 in this study, involve treatment of the horse with anthelmintics quarterly or every 60 days with different classes of anthelmintics in rotation. Some slow-rotation plans involve the use of a single anthelmintic for 1 yr followed by another single anthelmintic for the next year or two. These regimens typically also include the targeted use of boticides (ivermectin/moxidectin) or drugs to control tapeworms at specific times of the year.[18] Yearly rotation of anthelmintic class after testing for appropriate dewormer class has been suggested by other investigators.[18]

Most scientists agree that careful monitoring of parasite loads should be performed in equine management schemes. Early detection of resistance is equally important. In addition, regular FECR testing is highly recommended by many investigators to both monitor the status of the herd and to attempt to detect resistance at an early period.[7] Several researchers recommend the targeted approach to decrease the frequencies and amounts of anthelmintics used by monitoring FEC. Additionally, use of anthelmintic is recommended only after FEC reach a certain level. There is disagreement in the literature, however, as to what threshold of FEC should be reached before treatment is recommended.[19] An approach using both strategic and limited use of anthelmintics has also been suggested.[19]

Although it can vary with the different classes of anthelmintics, interval-dosing programs are designed to correlate doses of specific anthelmintic with the ERP.[20] Because of the great variation in patterns of fecal egg output in horses, monitoring individual efficacies is important, and custom plans may be indicated

Resistance to every main class of equine anthelmintic has been documented. Herd[21] recommended that complete reliance and emphasis on chemical anthelmintics to control parasite problems is no longer feasible because of the development of resistance, the shortening of ERP, and other warning signs. It is increasingly apparent that parasite resistance to current equine anthelmintics is an area of great concern. Parasitism in the horse can result in decreased performance and efficiency, diarrhea, rapid weight loss, loss of body condition, marked weakness, colic, and death in severe cases. It is widely believed that resistance in equine parasites will be an ever-increasing problem, and a greater understanding is needed to make important recommendations on rotational schemes.[22]

Future studies need to be done on different rotational schemes of fast versus slow rotation. This research only involved the fast rotation and is the first of its kind, and no definitive studies have been done on slow-rotation programs. Definitive studies are needed to examine the efficacies of the proposed plans. The correct use of anthelmintics and the development of rotational programs should be a priority in equine health worldwide.

Table 2. FECR in Horses on a Quarterly Rotational Program Sampled on Day 7 After Treatment

Treatment	Month	Number	FECR
Pyrantel pamoate	June	31	95.86%
Ivomectin + praziquantel	September	23	100%
Fenbendazole	December	21	97.84%
Moxidectin	March	26	100%

Implications

There are varying reports on which anthelmintic regimens should be used to achieve high levels of parasite control and prevent resistance. Experiments one and two indicated that neither frequent use (every 28 days) nor larger doses of FBZ will alleviate a FBZ-resistance problem in the horse. However, we showed that FBZ resistance in a herd can be reduced or broken by rotation of anthelmintic classes and that FBZ can be successfully implemented in a future rotational scheme subsequent to prior resistance. However, further research is needed to test and evaluate all aspects of rotation in the horse; specifically, comparisons of fast and slow rotation should be performed. Future studies will determine how we can slow the problem of parasite resistance and how each anthelmintic class can be used to the fullest efficacy in an equine population.

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[a]Safe-guard, Intervet, Millsboro, DE 19966.
[b]Panacur Powerpac Paste, Larvicidal Dose, Intervet, Millsboro, DE 19966.
[c]Animal Production Consulting, Lincoln, NE 68510.
[d]East Tennessee Clinical Research, Knoxville, TN 37921.
[e]Hanover Technology Pty Ltd., Rosewell, NSW, Australia.
[f]Texas A&M College of Veterinary Medicine, College Station, TX 77840.
[g]Statistix 8, Analytical Software, Tallahassee, FL 32317.
[h]Quest, Fort Dodge Animal Health, Fort Dodge, IA 50501.
[i]Zimectrin, Merial, Great Valley, NY 14741.
[j]Strongid Paste, Pfizer Animal Health, Exton, PA 19347.
[k]Zimectrin Gold, Merial, Great Valley, NY 14741.