Prevalence and clinical implications of anthelmintic resistance in cyathostomes of horses

Jaime L. Tarigo-Martinie, BS; Amy R. Wyatt, DVM; Ray M. Kaplan, DVM, PhD

Objective—To determine the prevalence and clinical implications of anthelmintic resistance in cyathostomes of horses.

Design—Prospective study.

Animals—80 horses on 10 farms in a 5-county region of northeast Georgia.

Procedure—On each farm, horses were stratified in descending order according to pretreatment fecal egg count (FEC), blocked into groups of 4, and then randomly assigned to 1 of 4 treatment groups: no treatment (controls), and treatment with pyrantel pamoate, fenbendazole, or ivermectin. Fecal samples were collected 24 hours prior to treatment and 2, 4, and 6 weeks after treatment for determination of FEC. Mean percentage of reduction in FEC was then calculated for each treatment group. For horses from each farm, the efficacy of each anthelmintic was categorized on the basis of mean percentage of reduction in FEC at 2 weeks after treatment (< 80% reduction = ineffective; 80 to 90% reduction = equivocal; and > 90% reduction = effective).

Results—Pyrantel pamoate was effective at reducing FEC in horses from 7 farms, ineffective in horses from 2 farms, and equivocal in horses from 1 farm. Fenbendazole was ineffective at reducing FEC in horses from 9 farms and equivocal in horses from 1 farm. Ivermectin was effective at reducing FEC in horses from all 10 farms.

Conclusions and Clinical Relevance—Results suggest that cyathostome resistance to fenbendazole is highly prevalent, and resistance to pyrantel pamoate is high enough to warrant concern. Resistance to ivermectin was not detected. On the basis of these data, it appears that ivermectin continues to be fully effective in horses. However, too few farms were used in this study to determine the prevalence of cyathostome resistance to ivermectin. Therefore, the efficacy of ivermectin should continue to be monitored closely. (*J Am Vet Med Assoc* 2001;218:1957–1960)

Cyathostomes (small strongyles) are recognized as the principal parasitic pathogen of horses.[1] Infections with cyathostomes usually cause a subclinical impairment of gastrointestinal tract function; however, infected horses may also have clinical signs such as decreased performance, weight loss, rough coat, and failure to meet full growth potential. Infection with cyathostomes can also result in a life-threatening clinical condition known as larval cyathostomosis, caused by synchronous emergence of numerous larvae from the intestinal mucosa. Horses with this condition usually have chronic diarrhea, which may be accompanied by colic, severe weight loss, and edema.

Anthelmintics are relied on as the primary means of nematode control in horses. There are 3 major classes of anthelmintics currently used to control nematodes in horses: **benzimidazoles** (**BZ**; fenbendazole, oxfendazole, and oxibendazole), tetrahydropyrimidines (pyrantel salts), and avermectin/milbemycins (macrocyclic lactones; ivermectin and moxidectin). Piperazine is a fourth drug class, but drugs of this class are rarely used.

When first introduced, all of these drugs had excellent efficacy against cyathostomes. However, it has now been documented worldwide that cyathostomes are becoming resistant to anthelmintics; this is gaining recognition as a serious concern in the health management of horses. Drug resistance in cyathostomes has been reported for phenothiazine, the BZ, piperazine, and pyrantel pamoate.[2] Of the 3 major classes of drugs, resistance to the BZ is the most prevalent and widespread, with reports from more than 21 countries documenting resistance. Resistance to 1 member of the BZ drug class confers resistance to other members within the same class,[3] with the exception of oxibendazole, which remains effective against BZ-resistant nematodes for a limited time.[4] Pyrantel-resistant cyathostomes have been reported in Louisiana,[5] Florida,[6] Kentucky,[a] Norway,[7] and Denmark.[8,9] The avermectin/milbemycins remain the only class of anthelmintic drugs currently used in horses for which resistance has not been reported. However, with growing reliance on these drugs, many parasitologists suspect that resistance is inevitable.[10]

With the increasing prevalence of anthelmintic resistance, it is important to monitor drug efficacy for proper management of cyathostome infections. It has been suggested that resistance to ivermectin will be prefaced by a reduction of the time required for eggs to reappear in feces following treatment (ie, **egg reappearance period [ERP]**).[11] Gaining knowledge of existing ERP in a geographic region by periodic monitoring may therefore be useful in early detection of development of resistance to ivermectin. Additionally, knowledge of ERP is useful for making recommendations concerning anthelmintic treatment intervals. The pur-

From the Department of Medical Microbiology and Parasitology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602.

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Address correspondence to Dr. Kaplan.

poses of the study reported here were to evaluate the efficacy of pyrantel pamoate, fenbendazole, and ivermectin in horses to determine the prevalence of resistance to these drugs in northeast Georgia, as well as evaluate the ERP associated with each drug.

Materials and Methods

Horses—Eighty horses on 10 farms with sufficiently high strongyle egg counts (≥ 150 **eggs per gram [EPG]**) were selected from a pool of 174 horses on 18 farms in Clarke, Oconee, Madison, Morgan, and Jasper counties in northeast Georgia. The farms included breeding and pleasure horse farms, and horses ranged in age from 1 to 24 years. Anthelmintic treatment programs on these farms included use of ivermectin exclusively and ivermectin rotated with pyrantel pamoate, fenbendazole, or both. Three farms had used moxidectin in the past, and 1 farm treated several horses daily with pyrantel tartrate.

Treatment groups—On each farm, horses were stratified in descending order according to pretreatment **fecal egg count (FEC)** and then blocked into groups of 4 horses, with the first block containing the 4 horses with the highest FEC, the second block having 4 horses with the next highest FEC, and so on. The number of blocks on each farm ranged from 1 to 4. Within each block, horses were randomly assigned to 1 of 4 treatment groups: group 1, untreated controls; group 2, treated with pyrantel pamoate[b] (6.6 mg/kg [3.0 mg/lb] of body weight, PO); group 3, treated with fenbendazole[c] (5.0 mg/kg [2.27 mg/lb], PO); and group 4, treated with ivermectin[d] (0.2 mg/kg [0.09 mg/lb], PO). All horses were given a paste formulation except for a few horses that received ivermectin liquid.[d] A girth tape was used to determine weight for calculation of dosages.

Parasitologic techniques—Fecal samples were collected per rectum or as freshly expelled feces off the ground at 24 hours prior to treatment and 2, 4, and 6 weeks after treatment. A modified McMasters test with a minimum sensitivity of 25 EPG was used to determine strongyle egg counts.[12]

Data analyses—Mean FEC for each treatment group was calculated at 0, 2, 4, and 6 weeks after treatment, using combined data from all farms. For individual farms, percentage of reduction in FEC was calculated for each horse at 2, 4, and 6 weeks after treatment by use of the following formula:

$$(\text{pretreatment EPG} - \text{posttreatment EPG})/(\text{pretreatment EPG}) \cdot 100$$

Mean percentage of reduction in FEC for all horses on a particular farm was then calculated for each drug. Treatments were categorized as effective, equivocal, or ineffective on each farm on the basis of mean percentage of reduction in FEC 2 weeks after treatment. Treatment was categorized as effective if FEC reduction was > 90%, equivocal if FEC reduction was between 80 and 90%, and ineffective if FEC was reduced by < 80%. The existence of drug-resistant cyathostomes was presumed on farms where drugs were declared ineffective. The ERP was defined as the time following treatment when mean FEC reached 20% or more of pretreatment values. Because this measure was irrelevant on farms where drugs were ineffective, ERP was not determined for those farms. A complete randomized block design[e] was used to test the hypothesis that FEC of all treatment groups were equal at week 0. **Confidence intervals (CI)** of 95% for percentage of prevalence of farms with anthelmintic-resistant cyathostomes were determined by use of tabulated values for binomial proportions.[13] Standard errors were calculated for observed FEC of each treatment group (n = 20 horses) and for mean percentage of reduction in FEC for each drug (n = 10 farms). Values of $P < 0.05$ were considered significant.

Results

Randomized assignment of horses to treatment groups was effective in creating groups with FEC that were not significantly different. Fecal egg counts of untreated control horses remained high throughout the study, confirming that events unrelated to anthelmintic treatment did not confound the results. Mean FEC from all horses combined (for each treatment group) were decreased from pretreatment values of 1,390, 1,173, and 1,150 EPG to 730, 231, and 0 EPG at 2 weeks after treatment for fenbendazole, pyrantel pamoate and ivermectin, respectively (**Fig 1**). Two weeks after treatment, percentage of reduction in FEC from individual farms varied from 82 to –114% for fenbendazole, 100 to 0% for pyrantel pamoate, and was 100% on all farms for ivermectin. Mean percentages of reduction in FEC for all 10 farms at 2 weeks after treatment were 35.5, 89.1, and 100% for fenbendazole, pyrantel pamoate, and ivermectin, respectively (**Fig 2**). The 3 drugs tested in this study were categorized as effective, equivocal, or ineffective on each farm based

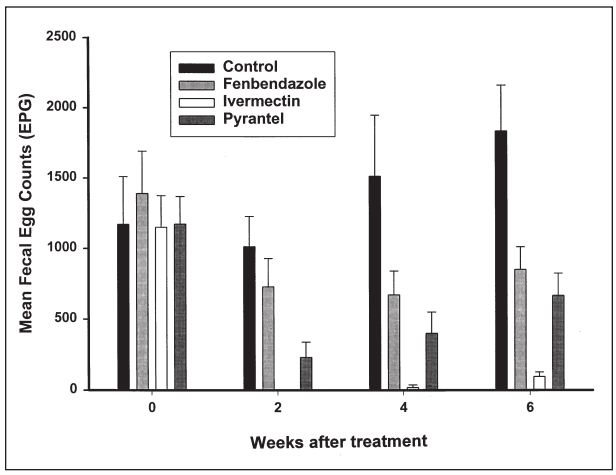

Figure 1—Mean fecal egg counts (FEC) of horses receiving fenbendazole, ivermectin, or pyrantel in each treatment group (n = 20 for each group) prior to treatment and 2, 4, and 6 weeks following treatment. EPG = Eggs per gram.

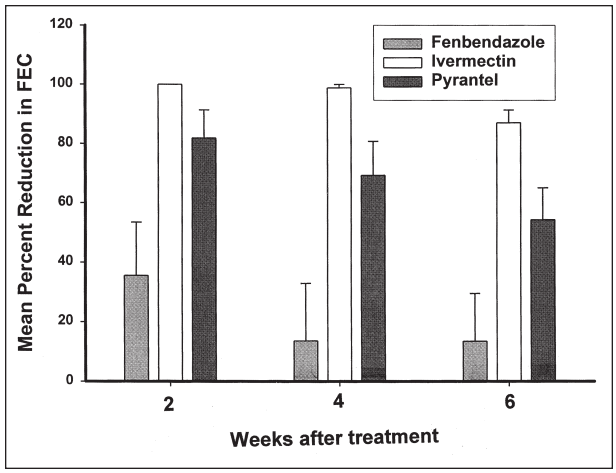

Figure 2—Mean percentage reduction in FEC for 10 farms at 2, 4, and 6 weeks following treatment with fenbendazole, ivermectin, or pyrantel.

Table 1—Treatment efficacy 2, 4, and 6 weeks after treatment with pyrantel pamoate, fenbendazole, or ivermectin, and percentage of farms with egg reappearance at 4 and 6 weeks

Treatment	Effective (> 90%)	Equivocal (80–90%)	Ineffective (< 80%)	Percentage of farms with egg reappearance*
Pyrantel pamoate				
Week 2	7	1	2	NA
Week 4	3	3	4	25
Week 6	1	1	8	75
Fenbendazole				
Week 2	0	1	9	NA
Week 4	0	0	10	100
Week 6	0	0	10	100
Ivermectin				
Week 2	10	0	0	NA
Week 4	10	0	0	0
Week 6	6	1	3	30

*Percentage of farms with egg reappearance at 4 and 6 weeks following effective or equivocal treatment efficacy at 2 weeks.
NA = Not applicable.

on the percentage of reduction in FEC 2 weeks after treatment (**Table 1**). On the basis of this data, mean percentages of farms with horses with cyathostomes that were resistant to anthelmintics were 90% (95% CI, 55.5 to 99.8%) for fenbendazole, 20% (95% CI, 2.5 to 55.6%) for pyrantel pamoate, and 0% (95% CI, 0 to 30.9%) for ivermectin.

Discussion

Results of the study reported here and similar recent studies performed in Florida[6,14] and Northern Europe[7,8,9] clearly indicate that resistance to fenbendazole is highly prevalent in cyathostomes in horses. In our study, treatment with fenbendazole was not effective in horses on any of the 10 farms. On the basis of these results, BZ anthelmintics (with the exception of oxibendazole, which was not tested) should not be used as a single-dose treatment without prior demonstration of efficacy. Efficacy of the 5-day double-dose regimen of fenbendazole for treatment of the mucosal larval stages of cyathostomes was not evaluated; therefore, no inferences can be made regarding the effectiveness of this dosing regimen.

Resistance to pyrantel pamoate was detected in horses from 2 of the 10 farms, and equivocal results were obtained from horses on 1 farm. In horses from 1 farm in which pyrantel was ineffective, there was a 0% reduction in FEC 2 weeks after treatment, indicating resistance of cyathostomes to pyrantel is high. This was the only farm in the study with a history of daily pyrantel tartrate use, which may have facilitated the development of resistance. Although the small number of farms examined may cause confidence limits for percentage of prevalence of resistance to be quite wide, results from this study and another in Florida[6] suggest that pyrantel pamoate be used judiciously and that resistance screening be performed before using pyrantel pamoate.

Ivermectin was effective in horses from all 10 farms, which is consistent with previous reports.[6,14-16] Therefore, it appears that use of this drug may be continued with confidence. However, because only horses from 10 farms were used in this study, it would be unlikely that resistance of cyathostomes to ivermectin would be detected if prevalence was low. To state with a high degree of statistical confidence that ivermectin resistance is not present would require demonstrating effectiveness on 72 of 72 farms. Therefore, the rare existence of ivermectin resistance cannot be excluded as a possibility. In recent years, control of cyathostomes in horses has become heavily dependent on use of ivermectin and moxidectin, chemically similar anthelmintics for which, to our knowledge, there is no reported resistance in parasites of horses. Historically, the development of resistance to anthelmintics in parasites of horses has been prefaced by the development of resistance in parasites of sheep and goats. Ivermectin-resistant nematodes of sheep and goats are becoming increasingly prevalent worldwide,[17] raising concerns about the development of ivermectin-resistant cyathostomes in horses.

The development of anthelmintic-resistant cyathostomes has been facilitated by an extensive reliance on the use of drugs solely for control of parasites. Therefore, strategies to decelerate further selection for resistance in cyathostomes and extend the lifetime of currently effective anthelmintics should be implemented whenever possible. This goal can best be achieved using epidemiologic principles of nematode control.[18] Properly timed treatments (which would vary, depending on drug used and geographic region) with anthelmintics combined with sound pasture management and horse husbandry can be effective in decreasing the number of drug treatments required as well as environmental contamination with cyathostome eggs and larvae. These would decrease the selection for drug resistance and minimize nematode infection in horses. Other suggestions for optimizing control of infection while minimizing the development of drug resistance include periodic testing of anthelmintic efficacy so that only effective anthelmintics are used, administering the correct dosage of anthelmintic, and rotating anthelmintic classes on an annual basis (as possible considering effectiveness).[18] The common practice of rotating drugs with each treatment does not appear to slow the development of resistance[19] and may actually increase the rate at which resistance develops by selecting for resistance to more than 1 drug simultaneously.[20] Unfortunately, other than pasture hygiene,[21,22] there are currently no effective means to control cyathostomes other than treatment with drugs. However, recent advances in biological control of nematodes by use of nematophagous fungi may soon help ease the reliance on drug-based control and allow for a more integrated approach to cyathostome management.[23] These fungi trap and destroy developing nematode larvae in the feces, thereby decreasing environmental contamination with infective larvae.[24]

It has been suggested that monitoring ERP may be valuable for early detection of developing resistance to ivermectin.[11] In our study, ERP was defined as the time point following treatment when mean FEC returned to 20% or more of pretreatment values to reflect the time at which an anthelmintic would be categorized as ineffective. In the 1960s, the ERP in horses treated with BZ drugs was between 6 and 8 weeks.[25] By the 1970s, frequent use of BZ had selected for cyathostomes with shorter ERP, and there were reports of high FEC at 6 weeks after treatment with BZ drugs.[20] When BZ

anthelmintics are still effective, the ERP for BZ is now considered to be approximately 4 weeks. In the 1980s, it was reported that ivermectin suppressed FEC for approximately 9 to 10 weeks after treatment,[26] but on many farms the ERP in horses treated with ivermectin now appears to be closer to 6 to 8 weeks. In a recent study in Florida,[6] the ERP was 6 and 8 weeks following treatment on 8 and 50% of the farms in which horses were effectively treated with ivermectin, respectively. In another study,[14] which was performed in the same region of Florida several years earlier, the ERP was 8 weeks following treatment on 27% of farms in which horses were effectively treated with ivermectin. In our study, which was performed in Georgia, the ERP was 4 and 6 weeks following treatment on 0 and 30% of farms in which horses were effectively treated with ivermectin, respectively. Although it appears that the ERP in horses is getting shorter following treatment with ivermectin, it is not possible to make direct comparisons between studies unless differences in pretreatment FEC and age of horses tested are controlled. Given these confounding factors, there are not enough data to draw any conclusions regarding what this shorter ERP may indicate in terms of evolving resistance.

The increasingly high prevalence of anthelmintic-resistant cyathostomes must be taken into account when designing nematode control programs for horses. It is strongly recommended that prior to using a BZ drug or a pyrantel salt, a small clinical trial be performed to rule out the presence of drug-resistant cyathostomes on that property. To perform this trial, horses should be assigned into balanced groups with regard to age, treated with the anthelmintics to be tested, and feces should be collected at the time of treatment and 2 weeks after treatment for determination of FEC. When more than 1 anthelmintic class is effective, an effort should be made to perform annual rotation. One anthelmintic should be used for an entire year and a second drug used the next. Acceptable exceptions to this guideline include single treatments for bots (using ivermectin/moxidectin) and tapeworms (using a double-dose regimen of pyrantel pamoate); these treatments can be added as needed in off-rotation years.

Because of the nature of the equine industry, in which horses often graze shared pastures with horses from diverse locations, transmission and widespread dispersal of resistant parasites is virtually assured. It is likely, therefore, that the prevalence of anthelmintic resistance on horse farms of 1 geographic region will not be greatly different from another. Considering these issues and the similarity in prevalence of resistance reported in this study and similar studies in Florida[6] and Denmark,[8] it appears that anthelmintic-resistant cyathostomes are prevalent and widespread. Therefore, annual monitoring of anthelmintic efficacy should be part of all health management programs of horses.

[a]Lyons, ET, Department of Veterinary Science, Gluck Equine Research Center, University of Kentucky, Lexington, Ky: Personal communication, 2000.
[b]Strongid Paste, Pfizer Inc, New York, NY.
[c]Panacur, Hoechst Roussel Vet, Somerville, NJ.
[d]Eqvalan, Merck and Co Inc, Rahway, NJ.
[e]PROC GLM, SAS version 6.12, SAS Institute Inc, Cary, NC.

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