Effectiveness of oxibendazole against benzimidazole-resistant strongyles in horses

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Abstract

Twenty-eight horses with a residual burden of strongyle eggs in the feces after treatment with mebendazole (MBZ) paste were treated with a suspension of either MBZ or oxibendazole (OBZ). Fecal samples were collected before and 14 days after these treatments. The number of strongyle eggs/g (epg) of feces for each horse was estimated using the Cornell-McMaster dilution and the Cornell-Wisconsin double centrifugation procedures. The epg for each horse was transformed using log (x + 1) and in an analysis of variance of the reduction in egg count for each horse on the logarithmic scale, there was a highly significant difference between the treatments. The mean epg was increased in the MBZ-treated horses and reduced in the OBZ-treated horses, but the reduction was only by 82% with an upper confidence limit of 89%. Subsequently, the horses were retreated with MBZ and OBZ suspensions without significant reduction in the mean epg for OBZ-treated horses.

Résumé

L'efficacité de l'oxibendazole contre les strongles résistants aux benzimedazoles chez le cheval

Vingt-huit chevaux présentant une infection résiduelle par des strongles après un traitement avec une pâte de mébendazole (MBZ) furent traités à nouveau avec une suspension de MBZ ou d'oxibendazole (OBZ). Les échantillons de fèces furent examinés avant et 14 jours après ces traitements. Le nombre d'œufs de strongles par gramme de fèces (opg) fut déterminé pour chaque cheval par la méthode de dilution Cornell-McMaster et par la méthode de double-centrifugation Cornell-Wisconsin. Les opg pour chaque cheval furent transformés par la formule log (x + 1). Une analyse de variance de la réduction du nombre d'œufs pour chaque cheval sur une échelle logarythmique démontra une différence hautement significative entre les deux traitements. L'opg moyen était augmenté chez les chevaux traités avec le MBZ et diminué chez ceux traités avec l'OBZ. Cependant, cette réduction n'était que de 82 % avec une limite de confiance supérieure à 89 %. Les chevaux furent traités à nouveau avec des suspensions de MBZ et OBZ sans qu'il n'y ait une réduction significative dans la réduction de l'opg chez les chevaux traités à l'OBZ.

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Introduction

Equine strongyles resistant to benzimidazole (BZ) anthelmintics have been recognized for some time now in some areas of the world including Canada (1-4). Strongyles that are resistant to a BZ compound have been reported to have side resistance to other BZ anthelmintics (2,3,5,6). Oxibendazole (OBZ), one of the newest of the BZ compounds, appeared to be unique in being highly effective against BZ-resistant strongyles (6-10). That level of effectiveness could not be demonstrated in horses in a field trial in Ontario (11). In that report, the preliminary findings on the usefulness of OBZ in horses known to be harboring resistant strongyles were presented. We report now in full the results of that study.

Materials and methods

The trial was conducted in October through December 1985 on a farm in southwestern Ontario with 28 Standardbred horses (27 mares and a stallion), 3 to 33 years of age. On day 0, each horse was given *per os* mebendazole (MBZ) paste, (Telmin, Pitman Moore, marketed by Moore, Thomson, Clinger, Mississauga, Ontario) at the dosage of 8.8 mg/kg of body weight. Fecal samples were taken from each horse immediately before treatment (day 0), and 14 days later (day 14) to determine the number of nematode eggs/g of feces (epg). For this determination, a 10 g sample of feces was used in the Cornell-McMaster dilution technique (12). When a sample from a horse contained less than 50 epg, another 5 g sample was analyzed by the Cornell-Wisconsin double centrifugation technique (13). A larval culture, consisting of 40 to 50 g of feces, was set up for each horse before and after treatment. After ten days, infective larvae were extracted from the culture by a Baermann procedure and from each culture 100 larvae or, if less, as many as possible that were available, were identified.

Horses were ranked by posttreatment epg so that the horse with the highest epg was at the top of the rank. On day 14, each member of the pair of horses at the top of the rank was randomly assigned to treatment with either MBZ suspension (Telmin) at the dosage of 8.8 mg/kg bodyweight, or with oxibendazole (OBZ) suspension (Anthelcide EQ, Norden Laboratories, Mississauga, Ontario) at a dosage of 10 mg/kg

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of body weight. Each treatment was given by nasogastric tube, which, before it was withdrawn from the horse, was flushed with about 300 mL of warm water. After the first two horses were treated, subsequent pairs in the rank order were similarly assigned to the treatment groups. Fecal samples were taken from all horses on day 28 for determination of an epg count as described previously. On day 42, the horses were treated again with the same anthelmintic as they had been treated with on day 14, but with anthelmintics from a different lot number. Fecal samples were taken from each horse on days 42 and 56 for an epg count.

Since the data were counts covering a wide range of values, the epg for each horse was transformed by log (x + 1). On days 14 and 28, the reduction in epg for each horse was calculated as 1 - antilog [log (x + 1) posttreatment - log (x + 1) pretreatment] (14). An analysis of variance (ANOVA) was conducted on the reduction of the count for each horse on the logarithmic scale. A point estimate for the % reduction for OBZ and a one-tailed upper confidence limit on the point estimate were determined. The analysis was repeated for data on days 42 and 56.

Results

Throughout the trial, all horses had strongyle eggs in the feces. The mean epg for the 28 horses prior to treatment with MBZ paste was 541.4 and 2 wk later it was 256.3. Only small strongyle larvae were found in the fecal cultures for all horses. The mean epg on days 14, 28, 42 and 56 for horses treated with suspensions of MBZ and OBZ are shown in Table 1. There was a highly significant ($p < 0.01$) difference between MBZ and OBZ treatments (Table 2). The first OBZ treatment decreased the epg significantly by 82% with an upper one-tailed ($\alpha = 0.05$) confidence limit of 89% (Table 3). The second OBZ treatment decreased the egg count by 37%, but this was not statistically significant (Table 3). The mean epg for horses treated with MBZ suspension increased following each treatment.

Discussion

In this field trial, horses with a residual strongyle epg after treatment with an MBZ paste had an 82% reduction in the mean epg following a further treatment with an OBZ suspension. When these horses were retreated with OBZ 28 days later, there was no significant reduction in the mean epg. These findings for OBZ are unlike higher reductions in epg (generally in the high 90% range) reported for horses with BZ-resistant strongyles (7–10). Another group of horses with a similar residual epg after treatment with MBZ paste had increases in the mean strongyle epg following each of two treatments with MBZ suspension.

There are no published surveys for BZ-resistant strongyles in horses in Canada, but in 1982 the first author (JODS) had examined ten farms in Ontario in which there had been frequent use of anthelmintics, and found that on four farms the horses had resistant strongyles. In 1984, the horses on the farm in the present study were shown to have BZ-resistant strongyles. Oxibendazole was not available commercially in Canada at the time of the study in October 1985. It had not been used on the farm previously and an

TABLE 1
Mean strongyle eggs/g feces and a range of values for horses that had been treated on day 0 with mebendazole (MBZ) paste and then on days 14 and 42 with a suspension of MBZ or oxibendazole (OBZ)

Day of trial	Treatment			
	MBZ (n = 14)		OBZ (n = 14)	
	Mean	Range	Mean	Range
14	263.1	1.0 - 1200	249.5	6.0 - 1200
28	415.9	5.0 - 1350	83.8	0.2 - 500
42	281.0	6.2 - 1550	90.4	1.4 - 700
56	557.8	2.8 - 1700	42.7	0.8 - 250

TABLE 2
Analysis of variance (ANOVA) of log-transformed reduction values for mebendazole and oxibendazole on days 28 and 56

Source	ANOVA		
	Degrees of freedom	Mean squares	
		Day 28	Day 56
Treatment	1	35.21[a]	11.38[a]
Error	27	1.29	1.05

[a]$p < 0.01$

TABLE 3
Mean log-transformed egg counts and % reduction at days 28 and 56 as a ratio of egg counts at days 14 and 42, respectively

Day	Treatment	Mean	Standard error	% reduction
14	MBZ	0.446	0.293	– 56
	OBZ	– 1.721	0.293[a]	82
42	MBZ	0.775	0.264[a]	– 117
	OBZ	– 0.457	0.264	37

[a]Significantly different from zero ($p < 0.01$)

assumption made was that the horses there had never been treated with the anthelmintic. The lower than expected response to the anthelmintic, therefore, was remarkable. Subsequently, an investigation of the history of the horses revealed that six of them had been imported from the USA in 1983 and six in 1984. Anthelcide EQ has been available in the USA since November 1980 and some of the horses in the present trial may have been treated there with that anthelmintic. Resistant strongyles have now been found in the USA in horses which had been treated repeatedly with OBZ (15-16). In one of these studies (16), OBZ was given every 2 mo over 6 yr and the percentage reductions in egg count in successive years were 96, 80, 81, 69, 43 and 37.

Oxibendazole is a highly efficient anthelmintic for horses (6, 17-19). It is particularly useful for foals, especially the paste formulation with ease of administration and its broad spectrum of activity including the roundworms, *Parascaris equorum* (9,20,21), and threadworms, *Strongyloides westeri* (7,9,20,22). However, we believe that BZ-resistant strongyles are common in Ontario.

A simple method to examine for the presence of resistant nematodes on a farm is to estimate the number of eggs in the feces of horses before treatment and 10 to 14 days later. Fecal samples from each horse, or at least 25% of the horses that run together as a group, should be examined. Following treatment with a highly effective anthelmintic, the egg count should be zero or close to it. An important cause of a lack of a significant reduction in egg count is the presence of resistant nematodes. Another cause may be that a suboptimal dose of the anthelmintic was administered. Such a dose may have contributed to the development of resistant worms. A determination of the fecal egg count before and after treatment, therefore, is useful for assessing the effectiveness of treatment and for monitoring the control program on a farm.

The procedures for testing for anthelmintic resistance in nematodes should be an integral component of the operation of any farm and should be conducted at least once a year. The continued use of an anthelmintic on a farm where there is a population of resistant strongyles increases the potential for parasitic problems on that farm. In the present trial, and following the first treatment with MBZ, the mean fecal strongyle egg count was reduced by about 50% because the treatment had removed the susceptible nematodes. Subsequently, and in spite of the horses receiving two further treatments with MBZ suspension the mean egg count increased because the horses now had only BZ-resistant strongyles. Moreover, the mean egg count increased markedly through the course of the trial so that at the end of the trial it was similar to that at the start 8 wk earlier. Readers may consult two reviews (23,32) for a discussion on the control of strongyles including the topic of drug resistance.

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