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The role of extense efficacy in the evaluation of anthelmintic resistance in horse strongyles

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Summary

The first experiment evaluated the reappearance of strongyle eggs in horses after treatment with five different anthelmintics. Fifty-eight horses with natural strongyle infections were selected and divided into six experimental groups, each with 9 – 10 horses (groups 1 – 5), and an untreated control group of 10 horses (group 6), respectively. The anthelmintics used were pyrantel pamoate (PP), ivermectin (IVM), albendazole (ABZ), oxibendazole (OBZ) and moxidectin (MOX). All anthelmintics were given orally at the recommended dose rate. In all groups faecal egg counts were performed 14, 28, 42, 56, 70, 84, and 98 days after treatment. The egg reappearance period after treatment with ABZ (eggs found in all 10 horses) and OBZ (eggs found in 4 horses) was 14 days, compared to periods of 42, 56, and 84 days after treatment with PP (eggs found in 2 horses), IVM (eggs found in 4 horses) and MOX (eggs found in 4 horses), respectively. The extense efficacy (EE) was 0 %, 14 days after treatment with ABZ compared to 60 % after treatment with OBZ.
In the second and third experiments the anthelmintic efficacy of PP and fenbendazole (FBZ) was investigated on two horse farms. Treatment with PP was very effective (avg. FECR: 99.1 %, EE: 73.9 %) on both farms while treatment with FBZ was significantly lower (avg. FECR: 87.6 %, EE: 2.3 %). The administration of multiple therapeutic doses of FBZ on one of the farms did not significantly increase the FECR or EE. In all the cases where the FECR was insufficient (< 90 %) the EE was very low (< 20 %). Therefore, this index could be used as complementary proof in the evaluation of anthelmintic treatment.

Key words: horses; strongyles; treatment; anthelmintics; anthelmintic resistance

Introduction

Although the use of anthelmintics to control the large strongyles in horses has been rather successful the same cannot be said for the small strongyles (cyathostomes), which are now recognised as the major intestinal parasites in horses (Love and Duncan, 1992).
In the 1960's the benzimidazole (BZ) class of anthelmintics were introduced as a remedy against intestinal nematodes (McKellar and Scott, 1990), where their high anthelmintic efficacy and improved safety compared to older products resulted in widespread and frequent use. The frequent use of thiabendazole (TBZ) resulted in the rapid development of parasites that were resistant to this compound (Drudge *et al.,* 1977). Despite the introduction of a number of improved BZ compounds resistance to this class of drugs is now problem on horse farms in many parts of the world (for review see Conder and Campbell, 1995). In addition, there have been reports of cyathostomes resistant to pyrantel (Chapman *et al.,* 1996; Craven *et al.,* 1998; Coles *et al.,* 1999; Dorny *et al.,* 2000) indicating an emerging problem for this drug class as well. There are no reports of cyathostomes resistant to the avermectin class of anthelmintics, but an extended and frequent use of these preparations will inevitably lead to resistance in this class (Lloyd and Soulsby, 1998) as it has in the other drug classes.
In Lithuania horse breeders have largely maintained a twice yearly use of anthelmintics and take every opportunity to alternate the anthelmintic used. Yet, in some farms benzimidazoles have been used to treat animals for five and more years, which implies that cyathostomes have probably developed a resistance to these drugs (Vyšniauskas and Kaminskaitė, 1999). In contrast, the application of pyrantel pamoate (PP) is making it's first steps in Lithuania.
Faecal egg count reduction (FECR) is the most widely

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used method for determination of anthelmintic resistance. While this gives an indication of the reduction in faecal egg excretion following treatment no information is provided as to the number of animals excreting eggs after treatment or the period of time for which egg excretion is inhibited. Unfortunately, most of the authors give no values of the extense efficacy (EE) of preparations showing the percentage of uninfected animals after treatment.

This work was designed to examine the efficacy of several anthelmintics on two Lithuanian horse breeding farms where BZs have been commonly used. For best results in estimating the anthelmintics efficiency we also determined the EE of anthelmintics and discussed the role of this index for this kind of investigations.

Materials and Methods

Horses

The experiments were conducted on two state owned Lithuanian breeding farms: SBF1 (SP UAB "Vilniaus žirgynas", Vilnius district) rearing about 200 horses and SBF2 (SP UAB "Nemuno žirgynas", Šilutė district) rearing about 300 horses.

A total of 144 horses were examined. The experiments included horses of both sexes from different breeds (Trakėnai, Žemaitukai, Arabian, thoroughbred saddle horses, Lithuanian heavyweight) with a large variation in age (range 2 – 23 years) and weight (300 – 780 kg). The bodyweights (BW) of horses were provided by the farm managers. The last anthelmintic treatment of selected horses had taken place 5 – 7 months prior to the start of the experiment.

Only horses whose faeces contained >200 strongyle eggs per gram were selected for the experiments. The horses were grouped so as to include intensively and less intensively invaded with helminths in each group and so as the number of eggs to be comparable between groups.

Anthelmintic history of the farms

Data about the use of anthelmintics in the examined farms is given in Table 1. Anthelmintics of the BZ class have been used on SBF1 since 1994. In the years 1994 to 2001 the anthelmintics of this class were used sixteen times, ivermectin (IVM) or moxidectin (MOX) was used five times and pyrantel pamoate (PP) was used three times. On SBF2, BZs were used nine times, IVM or MOX was used three times, and PP was used twice over the same time frame.

Anthelmintics

The following anthelmintics were administered orally to the horses: albendazole (ABZ; 10 mg/kg BW, Alben 50 % granules, Sanitas, Lithuania (LT)), FBZ (7.5 mg/kg BW, Fenben 50 % granules, Sanitas, LT), oxibendazole (OBZ; 10 mg/kg BW, Oxiben 50 % granules, Sanitas, LT), PP (19 mg/kg BW, Pirel 50 % granules, Sanitas, LT), IVM (0.2 mg/kg BW, Ivergen 1 % solution, Biogenesis, Argentina), MOX (0.4 mg/kg BW, Equest 2 % gel, American Cyanamid, New Jersey, USA). Ivermectin and MOX were administered by putting on the root of the tongue while the rest were fed with commercial fodder in the morning. The doses were calculated individually for each horse with respect to its weight + 10 % in order to avoid underdosing.

The Experiments

The first experiment was conducted on SBF1 in 1999 –

Table 1. Use of anthelmintics on two Lithuanian horse farms during the period 1994–2001

Year	Season	Horse-breeding farm	
		SBF1	SBF2
	Spring	n.d.	n.d.
1994	Autumn	FBZ	n.d.
	Spring	FBZ	n.d.
1995	Autumn	FBZ	n.d.
	Spring	ABZ	n.d.
1996	Autumn	ABZ	n.d.
	Spring	FBZ	FBZ
1997	Autumn	FBZ	FBZ
	Spring	FBZ	FBZ
1998	Autumn	FBZ	FBZ
	Spring	FBZ	OBZ
1999	Autumn	ABZ, FBZ, OBZ, PP, IVM, MOX	OBZ
	Spring	FBZ, OBZ, PP, IVM	OBZ
2000	Autumn	IVM	MBZ, OBZ, IVM
	Spring	FBZ, PP	PP
2001	Autumn	IVM	PP, IVM, MOX

n.d. – no data; FBZ – fenbendazole; ABZ – albendazole; OBZ – oxibendazole; MBZ – mebendazole; PP – pyrantel pamoate; IVM – ivermectin; MOX – moxidectin

2000 and included 58 horses. The horses were assigned to six (6) groups where the first group received PP (n=9), the second received ABZ (n=10), the third received OBZ (n=10), the fourth received IVM (n=9), the fifth received MOX (n=10) and the sixth was an untreated control group (n=10). The horses were kept indoor in individual pens throughout this experiment.

The second experiment was conducted on SBF1 in the spring of 2002. A total of 60 horses were divided into 5 groups of 12 animals. Horses from the first group were treated with PP, the second group received a single therapeutic dose of FBZ, the third received two therapeutic doses of FBZ 48 hours apart, and the fourth received three therapeutic doses of FBZ with a dosing interval of 48 hours. The control horses from the fifth group were not treated.

The third experiment was carried out on SBF2. The selected horses were divided into three groups. Eleven horses were treated with PP (group 1), 8 were treated with FBZ (group 2), and the remaining 7 were untreated control animals (group 3).

Parasitological examinations

Horse faeces were examined twice before the start of each experiment. The first examination included all horses on the farm, while only the faeces of the selected horses were examined at the second visit. After treatment, faeces were again examined: in the first experiment 14, 28, 42, 56, 70, 84, and 98 days after treatment, in the other experiments 14 days after treatment. In all cases fresh droppings were collected from the stall floor in the morning. The samples of faeces were stored in refrigerator at + 4°C. The stron-

Table 2. The mean number of strongyle eggs detected in the horses on SBF1 per gram faeces (EPG), the faecal egg count reduction (FECR), the number of horses excreting eggs and the extense efficacy (EE) of the drugs at different time intervals following treatment with several anthelmintics

Examination Terms	Days after treatment							
	0	14	28	42	56	70	84	98
Group 1 (N=9): Pyrantel Pamoate								
EPG	490	0	0	16	47	53	80	120
FECR (%)		100	100	95.9	90.7	85.7	81.0	68.6
No. Excreting	9	0	0	2	4	6	8	9
EE (%)		100	100	78	55	33	11	0
Group 2 (N=10): Albendazole*								
EPG	489	112						
FECR (%)		74.3						
No. Excreting	10	10						
EE (%)		0						
Group 3 (N=10): Oxibendazole								
EPG	478	14	34	42	108	122	204	234
FECR (%)		96.8	93.3	89.1	78.7	67.0	51.4	38.7
No. Excreting	10	4	4	7	9	10	10	10
EE (%)		60	60	30	10	0	0	0
Group 4 (N=9): Ivermectin								
EPG	424	0	0	0	27	80	162	171
FECR (%)		100	100	100	94.7	78.4	61.4	55.2
No. Excreting	9	0	0	0	4	6	8	9
EE (%)		100	100	100	55	33	11	0
Group 5 (N=10): Moxidectin								
EPG	439	0	0	0	0	0	20	52
FECR (%)		100	100	100	100	100	95.2	86.4
No. Excreting	10	0	0	0	0	0	4	10
EE (%)		100	100	100	100	100	60	0
Group 6 (N=10): Control								
EPG	501	436	510	386	506	370	420	382

* Samples were only taken at day 14 after treatment, since all horses excreted eggs at this time

gyle egg counts were performed by a modified McMaster method (Henriksen and Aagaard, 1976) with a sensitivity of 20 eggs per gram (EPG) of faeces. Each of the samples was examined thrice and an arithmetic mean was derived from the data obtained.
Using the data from coprological examinations before and after treatment the anthelmintic efficacy was evaluated as described by Coles *et al.* (1992).

$$FECR = 100\ (1 - Xt/Xc)$$

where FECR is the faecal egg count reduction, Xt is the arithmetic mean egg count of the treated horses and Xc is the arithmetic mean egg count of the control animals at the same time point after treatment. Anthelmintics were regarded as very efficient when the FECR was greater than 98 %, efficient when the FECR was 90 – 98 %, averagely efficient when the FECR was 80 – 89 %, and inefficient when the FECR was less than 80 % (Kassai, 1999).
Extense efficacy (EE) is the index showing the percentage of uninfected animals after treatment. The EE was calculated using the formula: EE = 100 (A/B), where A is the number of animals whose faeces contained no helminth eggs after treatment and B is the number of animals in the treatment group.

Statistical analysis
The dissimilarities between various groups of animals were evaluated by Fisher's t–test with a significance level of 0.05.

Results

In the first experiment (see Table 2) strongyle eggs were found in the faeces of all horses 14 days after treatment with ABZ and the FECR (74.3 %) indicated that this drug was ineffective. For this reason the experiment with this group of horses was discontinued.
Fourteen days after treatment with OBZ the faeces of four horses out of 10 contained solitary strongyle eggs. Despite this the drug was efficient with an FECR of 96.8 %. After 28 days the number of infected horses remained the same but the number of eggs increased reducing the FECR to 93.3 %. In subsequent examinations the number of infected horses and the number of eggs contained in faeces increased steadily until, 70 days after treatment, the faeces of all treated horses contained strongyle eggs (FECR 67 %). At the end of experiment (day 98) the FECR was only 38.7 %.

Table 3. The faecal egg count reduction (FECR) and extense efficacy (EE) of pyrantel pamoate (PP) and fenbendazole (FBZ) on two Lithuanian horse farms in the spring of 2002

Farm	Experimental group	Average number of strongyle epg		FECR %	No. Excreting eggs after treatment	EE (%)
		Before Treatment	14 days after treatment			
SBF1	Group 1 (N=12) PP	538	5	99.0	3	75
	Group 2 (N=12) FBZ one time	552	63	87.6	12	0
	Group 3 (N=12) FBZ two times	663	60	88.2	12	0
	Group 4 (N=12) FBZ three times	552	39	92.3	12	0
	Group 5 (N=12) Control	623	508		12	–
SBF2	Group 1 (N=11) PP	725	3	99.1	3	72.7
	Group 2 (N=8) FBZ	533	58	82.7	7	12.5
	Group 3 (N=7) Control	492	335		7	–

Examination of horses 14 and 28 days after treatment with PP showed that the FECR was 100 %. Forty-two days after treatment the faeces of two horses out of 9 had solitary strongyle eggs but the FECR remained high at 95.9 %. Eggs were found in the faeces of all PP treated horses 98 days after treatment and the FECR was 68.6 %.

Fourteen, 28 and 42 days after treatment with IVM horse faeces contained no strongyle eggs. After 56 days the faeces of 4 horses out of 9 contained strongyle eggs. Nevertheless, the FECR at this stage remained high (94.7 %). In subsequent examinations the number of strongyle eggs increased together with the growing number of infected horses. Eggs were found in the faeces of all IVM treated horses 98 days after treatment and the FECR was 55.2 %.

Strongyle eggs did not appear in the faeces of horses treated with MOX for more than two months. Eighty-four days after treatment faeces of 4 of the 10 horses had strongyle eggs but the FECR remained high (95.2 %). Eggs were found in the faeces of all MOX treated horses 98 days after treatment and the FECR was 86.4 %.

Fourteen days after treatment the EE of ABZ was 0 %, whereas the EE of OBZ was 60 % and the EE of PP, IVM and MOX were 100 % at this stage. By 98 days after treatment the EE of all drugs was 0 %. The FECR of PP was lower than that of MOX but higher than that of IVM and OBZ.

The data of the second and third experiments are presented in Table 3. There was no significant difference in the efficacy of single, double and triple FBZ treatments on SBF1. However the single (FECR 87.6 %) and double (FECR 88.2 %) doses were rated as being of average efficacy, whereas triple dosing (FECR 92.3 %) was rated as efficient. Fourteen days after treatment all the horses from all three FBZ groups excreted strongyle eggs (EE 0 %). In comparison, only 3 of 12 horses (EE 75 %) treated with PP excreted strongyle eggs in the faeces 14 days after treatment (FECR 99 %).

On SBF2 the faeces of 7 out of 8 horses (EE 12.5 %) contained helminth eggs 14 days after treatment with a single FBZ dose. The obtained results evaluated this anthelmintic as one of average efficiency (FECR 82.7 %). At the same time PP was very efficient (FECR 99.1 %) on this farm though solitary strongyle eggs were found in faeces of 3 out of 11 horses (EE 72.7 %).

The summarised results of experiments 2 and 3 revealed that 6 of 23 horses treated with PP excreted eggs 14 days after treatment (FECR 99.1 %; EE 73.9 %), whereas 43 out of 44 horses treated with FBZ excreted eggs at this time (FECR 87.6 %; EE 2.3 %).

Discussion

The first experiment examined the egg reappearance period (ERP) of several drugs and determined the egg count reduction attributable to these drugs over an extended time period post-treatment. Fourteen days after treatment all horses treated with ABZ and 4 of the 10 horses treated with OBZ excreted eggs. Previous studies have shown that the ERP of BZ compounds is 4 – 6 weeks (Drudge and Lyons, 1966; Herd *et al.,* 1981; Piché *et al.,* 1991) although repeated treatment has been shown to reduce this period (Herd *et al.,* 1981). The much shorter ERP seen in the present study suggests the presence of BZ-resistant worms. The ERPs of 42, 56 and 84 days for PP, IVM and MOX, respectively, were similar to those previously reported (Piché *et al.*, 1991; DiPietro *et al.,* 1997).

With an extending assortment, widening spectrum and rising efficiency of anthelmintics their evaluation standards become stricter. As recently as the 1980s an anthelmintic was described as very efficient if it reduced the FECR by more than 90 %, moderately efficient if the FECR was 75 – 90 %, of poor efficacy if the FECR was 50 – 75 % and ineffective if the FECR was below 50 % (Marriner and Armour, 1985). More recently the world association for the advancement of veterinary parasitology (WAAVP) has recommended that an FECR below 90 % is indicative of anthelmintic resistance in horses (Coles *et al.,* 1992) while others (Craven *et al.,* 1998) advocate an FECR below 95 % as indicative of anthelmintic resistance.

The poor efficacy of ABZ (FECR 74.3 %) and average efficacy of FBZ (FECR 87.6 %) on SBF1 and the average efficacy of FBZ (FECR 82.7 %) on SBF2 suggest the presence of BZ resistance (FECR < 90 %) on both farms despite the rotational use of anthelmintics in recent years on both farms. Oxibendazole has previously been reported to maintain efficacy against BZ resistant cyathostomes (Drudge *et al.,* 1981a,b; Webster *et al.,* 1981; Lyons *et al.,* 1996) and this was again indicated in the present study where OBZ was highly efficient on SBF1. Since, even with infrequent treatment, the majority of helminths in horses are cyathostomes (Eydal and Gunnarsson, 1994) and all reports of BZ resistance in horses are due to cyathostome species (Conder and Campbell, 1995) it is believed that the farms investigated in this study were infected with small strongyles resistant to BZ compounds. However, no larval cultures were performed to verify this.

Despite relatively high FECR values (see Table 3) all horses treated in the present study with either 2 or 3 doses of FBZ at intervals of 48 h still excreted eggs 14 days after treatment. Repeated treatment with BZs at short intervals (approx 12 hours) has previously been shown to increase drug efficacy, even against BZ resistant parasites (Duncan *et al.,* 1980; Sangster *et al.,* 1991), but there was no significant difference between the single and multiple treatments on SBF1 in this study. This is most likely due to the relatively long treatment interval in the current study.

The use of PP was very effective on both farms with FECR values of 99.3 %. This suggests that PP is an efficient substitute to benzimidazoles, but use of this product should be strictly regulated as there are reports of nematodes that are resistant to this compound (Chapman *et al.,* 1996; Craven *et al.,* 1998). Ivermectin and MOX were also highly effective and while there are no reports of field resistance to these drugs in horses the frequent use of these products will inevitably lead to the development of resistance in this class as well (Lloyd and Soulsby, 1998).

The investigation of anthelmintic resistance cannot, however, be confined to calculations of the FECR of the anthelmintic. An FECR can, for example, be significantly influenced by treatment failure in one of the treated animals, but this does not reflect the presence of anthelmintic resistance in the herd. It is therefore important to know the number of horses who continue to excrete eggs after treatment. An assumption has been made already that EE might be one of the indicators for proving of anthelmintic resistance (Vyšniauskas and Kaminskaitė, 1999). This is in agreement with the findings of Bauer (1994), who emphasised the significance of EE for evaluation of anthelmintic efficiency in dogs and cats.

Treatment with either ABZ or FBZ on the two farms resulted in low EE values (< 20 %) 14 days after treatment. In contrast the EE values for OBZ (60 %), PP (72.7 – 100 %), IVM (100 %) and MOX (100 %) were considerably higher.

While it has been reported that one anthelmintic may be used successively twice a year without inducing resistance (Tarigo-Martinie *et al.,* 2001) we recommend the use of IVM or MOX (effective against gastrophiles and ectoparasites) for autumn treatment followed by the use of anthelmintics from the tetrahydropyrimidine class, organophosphorous compounds or OBZ (if effective) for spring treatment. Although non-chemical measures of intestinal nematode control are being widely discussed (Herd, 1986; Baudena *et al.*, 2000), anthelmintics continue to be the main means of control.

Testing for anthelmintic resistance is an important component of an effective treatment strategy and measuring the extense efficacy of treatment in addition to the FECR will aid in the diagnosis of resistance.

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