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A Field Study on the Effect of Some Anthelmintics on Cyathostomins of Horses in Sweden

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ABSTRACT

The objective of the study was to investigate different aspects on the efficacy of three anthelmintics on cyathostomin nematodes of Swedish horses. A faecal egg count reduction (FECR) test was performed on 26 farms. Horses were treated orally with recommended doses of ivermectin, pyrantel pamoate or fenbendazole. Faecal samples were collected on the day of deworming and 7, 14 and 21 days later. No resistance was shown against ivermectin; the FECR was constantly >99%. The effect of pyrantel was assessed as equivocal in 6 farms 14 days after treatment; the mean FECR was 99%. As many as 72% of the fenbendazole-treated groups met the criteria for resistance; the mean FECR was 86%, ranging from 56% to 100%. A re-investigation of two farms where pyrantel resistance had been suspected clearly revealed unsatisfactory efficacy of pyrantel on one of these farms; the FECR varied from 72% to 89%. Twenty-six of the horses previously dosed with pyrantel or fenbendazole, and which still excreted ≥150 eggs per gram of faeces 14 days after treatment, were dewormed with ivermectin and fenbendazole or pyrantel in order to eliminate the remaining cyathostomins. A total of 13 cyathostomin species were identified from horses that initially received fenbendazole and seven species were identified from pyrantel-treated individuals. The egg reappearance period (ERP) following treatment with ivermectin and pyrantel was investigated on two farms. The shortest ERP after ivermectin treatment was 8 weeks and after pyrantel was 5 weeks. We conclude that no substantial reversion to benzimidazole susceptibility had taken place, although these drugs have scarcely been used (<5%) in horses for the last 10 years. Pyrantel-resistant populations of cyathostomins are present on Swedish horse farms, but the overall efficacy of pyrantel is still acceptable.

Keywords: anthelmintic resistance, cyathostomins, egg reappearance period (ERP), faecal egg count reduction test (FECRT), horse

Abbreviations: ECR, egg count ratio; FECRT, faecal egg count reduction test; FEC, faecal egg count; epg, eggs per gram of faeces; ERP, egg reappearance period; LC, the lower 95% confidence limit

INTRODUCTION

Strongyle nematodes of the tribe Cyathostominea, commonly called cyathostomins, parasitize the large intestine of virtually all horses. Although more than 50 species of cyathostomins have been described in equids, only six were found to represent more than 90% of the cyathostomin population in Swedish horses (Osterman Lind *et al*., 2003). Clinically, cyathostomins are considered the principal helminth pathogen of the horse (Love *et al*., 1999) and accordingly extensive resources are directed towards their control. On Swedish horse farms, endoparasite control is usually based on drug treatment alone, but in some establishments such treatment is combined with some form of grazing management.

Benzimidazole resistance in populations of cyathostomin nematodes of the horse has been reported from many countries around the world (Kaplan, 2002); the first finding was only 4 years after thiabendazole had been introduced onto the market (Drudge and Lyons, 1965). Despite the widespread use of pyrantel compounds against horse nematodes for some 20 years, resistance was not reported until 1996 on a Thoroughbred stud farm in Louisiana, USA (Chapman *et al*., 1996). Since then, pyrantel resistance has also been documented in Denmark (Craven *et al*., 1998), Norway (Ihler, 1995), the UK (Coles *et al*., 1999) and southern USA (Woods *et al*., 1998; Tarigo-Martinie *et al*., 2001; Kaplan *et al*., 2004). In general, very little information is available on which cyathostomin species are resistant.

In view of emerging pyrantel resistance and reports of shorter egg reappearance periods (ERP) for ivermectin (Tarigo-Martinie *et al*., 2001; Little *et al*., 2003), it is essential to monitor the efficacy of the anthelmintics currently used. The great majority of the anthelmintic resistance studies performed in horse herds have been based on faecal egg count reduction test (FECRT). This procedure is easy to conduct, but to make valid judgements it initially requires many horses with positive egg counts. Anthelmintic resistance can also be detected by various *in vitro* methods. One test that has been frequently used in sheep flocks is the larval development assay (LDA) DrenchRite (Horizon Technology, 1996). This test has also recently been evaluated for cyathostomin infections in the horse (Tandon and Kaplan, 2004; Osterman Lind *et al*., 2005), but it was concluded that the test was not sufficiently reliable to be useful as a general diagnostic tool. Hence, for the next few years it is likely that FECRT will remain the most common method for the detection of anthelmintic resistance in cyathostomin populations.

The aims of this study were (1) to investigate the occurrence and the levels of anthelmintic resistance against ivermectin, pyrantel and fenbendazole by FECRT in 26 horse establishments in Sweden; (2) to determine the ERP following treatment with ivermectin and pyrantel; and (3) to identify the species composition in drug-resistant cyathostomin populations.

MATERIALS AND METHODS

Selection of farms and conduct of the FECRT

Horses from 40 farms in various parts of Sweden were screened by analysis of faecal samples. No treatment with anthelmintics had been undertaken for at least 8 weeks prior to sampling. The number of strongyle eggs per gram of faeces (epg) was determined by a modified McMaster technique, with a lowest detection level of 50 epg. Among the screened herds, 17 studs, 6 riding schools and 3 trotting stables (a total of 334 horses) participated in a FECRT, which was performed in April–May 2000. Fourteen farms that participated in the screening procedure were excluded from the FECRT because too few horses had sufficiently high FEC values. On each farm, individuals with $\geq$200 epg were allocated to three comparable treatment groups according to age and strongyle egg output. The groups, each comprising 3–8 horses, were treated according to the manufacturers' recommendations

by a local veterinarian using commercially available anthelmintic pastes in the following way: group 1 with 0.2 mg ivermectin (Ivomec, Merial, Lyon, France) per kg body weight (bw); group 2 with 19 mg pyrantel pamoate (Banminth, Pfizer Aps, Animal Health, Ballerup, Denmark); group 3 with 7.5 mg fenbendazole (Axilur, Invervet International BV, Boxmeer, The Netherlands). The body weights of the horses were estimated by the use of a girth tape.

Faecal samples were collected and sent to the laboratory of SWEPAR for analysis on day 0 (the day of treatment) and then again 7, 14 and 21 days post treatment. Pooled samples from day 0 were incubated at 25°C for 10–14 days. Third-stage larvae were then collected by means of the Baermann procedure and identified either as *Strongylus* spp. or Cyathostominae spp.

The faecal egg count reduction (FECR) was calculated on geometric means of FEC values (epg) using a method described by Bjørn and colleagues (1991):

- Change in faecal egg output of each horse was calculated as the egg count ratio (ECR) according to the formula: $\mathrm{ECR} = \ln((\mathrm{EPG_{p.t.}}+1)/(\mathrm{EPG_{d0}}+1))$, where $\mathrm{EPG_{p.t}}$ is the EPG value obtained post treatment and $\mathrm{EPG_{d0}}$ is the EPG on day 0.
- The FECR of the treatment group was calculated by back-transformation of the mean of ECRs: $\mathrm{FECR} = \{1-\mathrm{e}\hat{}[1/n(\sum \mathrm{ECR})]\} \times 100\%$, where the constant e is the base of natural logarithms.
- The lower 95% confidence limit of FECR (LC) was calculated for each treatment group: $\mathrm{LC} = 1-\exp(1/n \times (\sum \mathrm{ECR}) + \mathrm{CI}) \times 100\%$, where $\mathrm{CI} = \mathrm{t_{0.5.df}} \times \mathrm{SD(ECR)}/n^{0.5}$, $t_{0.5.\mathrm{df}}$ is the *t*-value at 5% level at $\mathrm{df} = n - 1$ degrees of freedom, SD(ECR) is the standard deviation in ECR, and n is the number of horses in the group.

Criteria for resistance

For ivermectin and fenbendazole, resistance was considered when the FECR was <95% and the lower 95% confidence limit (LC) of the reduction was <90% (Anonymous, 1989). Pyrantel resistance was considered when the FECR was <90% and the LC <80% (Pook *et al.*, 2002). If only one of the conditions was met, resistance was suspected. Comparison of the FECR obtained on different sampling days was performed by Kruskal–Wallis non-parametric test (Stata®), with the significance level set to 0.05. Comparison between FECR data in this study and a study performed in 1986 was made by a *t*-test.

Pyrantel specific FECRT

Two farms where pyrantel resistance had been suspected in 2000 were re-investigated in 2002–2004. One farm (no. 25) had approximately 40 school horses, performance horses and boarding horses, which were of mixed breeds and ages. The turnover rate of horses on this establishment was rather high; approximately 1/3 of the horses had been replaced every second year. For 6 years prior to the study, ivermectin had been the main anthelmintic used, sometimes rotated with pyrantel within the same season. The anthelmintic treatments

had been performed 3–4 times per year, mainly in the summer season. FECR tests were performed in October 2002 ($n = 9$), May 2003 ($n = 13$) and May 2004 ($n = 9$). Horses excreting ≥200 strongyle eggs per gram of faeces were treated with 19 mg pyrantel pamoate per kg bw and faecal samples were taken 14 days later. In 2003, two horses on this farm were re-treated with pyrantel 7 days after the first pyrantel deworming. In addition, expelled cyathostomins were collected and identified from another 4 horses one day following ivermectin administration, 7/14 days after the initial pyrantel treatment. The horses were not dewormed between the studies in 2002 and 2003. Between 2003 and 2004 moxidectin had been used.

The other farm (no. 3) had approximately 15 Swedish standardbred horses, of which the majority were younger than 5 years. Every year, 2–4 foals were bred and occasionally new horses were introduced to the herd. For several years prior to the study, ivermectin and pyrantel had been equally used, totalling 3–4 treatments per year. The FECRT was performed only in 2003 ($n = 9$) as most horses excreted too few eggs in 2004. The horses had been dewormed with moxidectin in the summer of 2003.

Egg reappearance period (ERP) for ivermectin and pyrantel

On another two farms, one stud in the south (no. 8) and one in the north (no. 12) of Sweden, an ERP study was performed in April–May 2003. Since fenbendazole resistance had been shown on both these farms, the ERP was examined only for ivermectin and pyrantel. On each farm, horses with FEC ≥200 epg were allocated to two comparable treatment groups of 8–10 horses each according to FEC and age. The mean ages were 6 years (no. 12 Pyr), 8 years (no. 8 Pyr), 6 years (no. 12 Ivo) and 9 years (no. 8 Ivo). The groups were treated according to the manufacturers' recommendations with ivermectin or pyrantel pamoate, respectively. Untreated control groups of 3–5 horses were also included on each farm. Faecal egg counts were performed on the day of treatment, 2 weeks after treatment and then on a weekly basis until 3 weeks after the mean FEC for a treatment group had exceeded 100 epg. The ERP was defined as the number of weeks elapsing from the day of anthelmintic treatment until the arithmetic mean of strongyle epg exceeded 100.

Identification of expelled cyathostomins

On 14 farms that were included in the FECRT, horses with ≥150 epg 14 days after treatment with fenbendazole or pyrantel were dewormed again on day 21 with the two anthelmintics they had not been treated with initially on day 0 (ivermectin and fenbendazole/pyrantel). The horse owners collected approximately 200 g of faeces per horse 24–30 h after the second treatment. These samples were fixed in 5% formalin and subsequently washed over a 150 μm screen and examined for cyathostomin worms. Following clarification in 80% phenol in glycerin, sexually mature worms were identified to species level according to Dvojnos and Kharchenko (1994). The permutation method of the Fisher exact test (Roff and Bentzen, 1989) was used for comparing the frequency distributions of expelled worms.

RESULTS

FECRT on 26 farms

FECs and larval cultures showed that 95–100% of the eggs recovered were cyathostomin nematodes. Eight farms (31%) had at least one horse excreting eggs of *Strongylus vulgaris*. Not surprisingly, the widest 95% confidence intervals were observed in the smallest fenbendazole and pyrantel treatment groups, i.e. those that consisted of 3–4 horses. Forty-eight per cent of these groups showed the same result on all three sampling occasions, and 75% of the groups consisting of 6–8 horses.

Ivermectin. No resistance was shown against ivermectin; the FECR was >99% on all three sampling occasions. The lowest LC calculated for the ivermectin-treated groups was 95%, except for one group of three horses, where it was negative owing to one horse that had 100 epg on day 21.

Pyrantel. The FECR for pyrantel varied from 95% to 100%, with an overall mean of 99% (Table I). Thus, according to the definition used in this study, resistance was not declared on any of the farms. However, because the LC was <80%, nine farms were assessed as suspected resistant at least on one of the three sampling occasions. The six treatment groups that consisted of 6–8 horses were all classed as susceptible all three sampling days.

Fenbendazole. The FECR following fenbendazole treatment revealed that resistance was widespread in the horse herds studied (Table II). Fourteen days post treatment, 72% of the farms met the criteria for resistance and the mean FECR was 86%, ranging from 56% to 100%. Because of the LCs, no farm was declared as susceptible. Nevertheless, the mean FECR was significantly higher on day 7 (93%) than on days 14 (86%) and 21 (84%).

FECRT of pyrantel on two farms

The follow-up of two farms confirmed that pyrantel resistance existed on farm no. 25 (Table III). On this farm similar results were obtained for three consecutive years; the lowest reduction was observed in October 2002 (72%). In 2003, two horses that were retreated with pyrantel 7 days after the first pyrantel treatment still excreted 250 and 400 epg, respectively, 7 days later. On farm no. 3, the mean FECR was 93% but, owing to an LC of 65%, the farm was still declared as suspected resistant.

ERP for ivermectin and pyrantel

The mean FEC values on the day for treatment were: 2169 epg (no. 12 Pyr), 1140 epg (no. 8 Pyr), 1894 epg (no. 12 Ivo) and 1194 epg (no. 8 Ivo). Farm no. 8 had a mean FEC of 107 epg already 3 weeks after the pyrantel treatment, but subsequently the mean declined slightly and remained below 100 epg for 3 weeks (Figure 1). From 6 weeks and onwards the mean FEC steadily increased in this group. Hence, for pyrantel the ERP was assessed as 6 weeks on farm no. 8 and as 5 weeks on farm no. 12. The ERP for ivermectin was 10 weeks on farm no. 8 and 8 weeks on farm no. 12. There were no significant changes in mean FEC values over the sampling period in the control groups.

TABLE I
Faecal egg count reduction test following treatment with pyrantel in 23 horse herds in Sweden

			Day 0		Day 7			Day 14			Day 21		
Farm no.	*n*	Mean age (years)	Arith. mean[a] epg	Geom. mean[a] epg	FECR (%)	LC[b]	R/S[c]	FECR (%)	LC	R/S	FECR (%)	LC	R/S
1	6	5	850	623	99	96	S	100	97	S	100	99	S
2	4	3	563	494	100	99	S	100	99	S	100	97	S
3	3	5	683	631	99	57	SR	98	−689	SR	96	−1447	SR
4	5	6	700	567	99	92	S	98	61	SR	95	−3	SR
6	4	11	1100	806	90	65	SR	98	81	S	96	11	SR
8	7	8	743	660	100	100	S	100	99	S	100	99	S
9	8	3	988	825	100	100	S	100	97	S	100	96	S
10	8	2	981	806	99	97	S	99	96	S	99	97	S
11	4	3	963	935	99	67	SR	99	70	SR	ND[d]		
12	6	3	1142	997	99	97	S	99	95	S	100	98	S
13	5	4	640	546	99	91	S	99	97	S	99	97	S
14	5	6	1170	879	99	87	S	100	99	S	98	72	SR
15	5	7	1280	1118	99	90	S	99	73	SR	99	67	SR
16	4	7	475	405	100	99	S	98	89	S	99	80	S
17	3	3	1283	867	ND			100	98	S	100	99	S
18	4	3	1050	884	100	100	S	99	95	S	100	99	S
19	4	4	1675	1549	99	88	S	ND			100	95	S
20	6	4	817	648	100	99	S	100	100	S	100	100	S
21	3	11	1250	1037	ND			100	48	SR	100	94	S
22	3	11	1167	1067	100	−645	SR	99	96	S	96	−108	SR
23	3	4	817	777	100	100	S	100	100	S	100	86	S
24	4	10	1275	1221	99	93	S	100	99	S	100	94	S
25	4	12	800	670	99	−173	SR	99	41	SR	99	85	S
Mean	6	5	974	827	99			99			99		

[a]Arith. mean, arithmetic mean; Geom. mean, geometic mean
[b]LC, lower 95% confidence limit
[c]R, resistance (FECR <90%; LC <80%); S, susceptibility; SR, suspected resistance
[d]ND, no data obtained

Species identification

From 26 horses that had at least 150 epg 14 days after the initial treatment with fenbendazole or pyrantel, a total of 1892 specimens belonging to 13 cyathostomin species were identified. In addition, there were larval stages (15%), which could not be identified to species level. Thirteen species were recovered from 19 horses that had initially been treated with fenbendazole and 10 species from 7 horses that had initially been treated with pyrantel (Figure 2). Nematodes identified from the fenbendazole-treated horses consisted of 963 (66%) adults, 270 (18%) juveniles and 236 (16%) larvae. Correspondingly, 248 (60%) adults, 125 (30%) juveniles and 50 (12%) larvae were identified from the pyrantel-treated horses. The frequency of *Cylicocyclus nassatus* and *Cyathostomum catinatum* differed significantly

TABLE II
Faecal egg count reduction test following treatment with fenbendazole in 26 horse herds in Sweden

			Day 0		Day 7			Day 14			Day 21		
Farm no.	*n*	Mean age (years)	Arith. mean[a] epg	Geom. mean[a] epg	FECR (%)	LC[b]	R/S[c]	FECR (%)	LC	R/S	FECR (%)	LC	R/S
1	5	5	1050	872	89	59	R	95	−160	SR	95	−137	SR
2	3	1	567	541	94	−11	R	58	−97	R	66	−5	R
3	4	6	563	482	80	−5	R	74	17	R	87	−669	R
4	5	6	710	656	92	−50	R	56	−62	R	65	−1284	R
5	3	9	1067	816	73	30	R	94	−70153	R	81	−359	R
6	4	11	1138	1088	99	66	SR	90	67	R	97	−218	SR
7	3	12	567	505	98	−854	SR	97	−176	SR	87	76	R
8	6	9	821	695	93	82	R	82	63	R	68	−23	R
9	7	3	1221	901	99	76	SR	98	83	SR	99	88	SR
10	8	2	656	610	87	55	R	77	53	R	78	55	R
11	4	3	1275	1039	97	−252	SR	85	−41	R	ND		
12	6	3	1733	1428	97	15	SR	91	−49	R	91	−53	R
13	5	5	600	548	95	7	SR	76	5	R	57	−16	R
14	3	7	1150	957	92	66	R	76	2	R	74	−39	R
15	6	8	1425	1314	95	62	SR	88	60	R	77	56	R
16	5	6	390	345	94	41	R	83	34	R	91	−10	R
17	3	3	683	639	97	−804	SR	100	75	SR	98	−205	SR
18	4	3	1088	669	91	42	R	84	49	R	95	−31	SR
19	4	4	1525	1166	84	4	R	ND[d]			65	−44	R
20	5	5	1310	1062	93	−69	R	87	59	R	85	−21	R
21	4	9	1150	923	ND			96	47	SR	94	43	R
22	4	10	1013	983	95	55	SR	79	34	R	89	59	R
23	3	5	1117	981	95	58	SR	94	46	R	90	46	R
24	5	17	1340	1276	99	88	SR	98	80	SR	96	89	SR
25	6	10	800	688	95	65	SR	93	58	R	85	69	R
26	3	6	817	497	99	25	SR	99	−155	SR	94	−2903	R
Mean	5	6	991	834	93			86			84		

[a]Arith. mean, arithmetic mean; Geom. mean, geometic mean
[b]LC, lower 95% confidence limit
[c]R, resistance (FECR <95%; LC <90%); S, susceptibility; SR, suspected resistance
[d]ND, no data obtained

between horses initially treated with fenbendazole and horses initially treated with pyrantel ($p < 0.0001$).

DISCUSSION

Based on data from the 26 farms presented here, it can be concluded that pyrantel resistance did not appear to be a widespread problem in Sweden. Also, the ERP for pyrantel was

TABLE III
Faecal egg count reduction test for pyrantel on farm no. 25 in 2002–2004

Testing year	Horse ID	Age (years)	EPG day 0	EPG day 14	FECR and assessment[a]
Oct 2002	1	7	1600	1700	
	2	20	700	50	
	3	7	750	300	
	4	18	1400	2000	
	5	19	850	50	
	6	2	1050	1500	
	7	18	350	550	
	8	19	300	0	FECR: 72%
	9	11	550	300	LC: −38%
Mean		13	839	717	R/S: R
May 2003	1	8	1100	0	
	2[b]	21	1050	0	
	3[b]	8	850	0	
	4	5	550	350	
	5	3	450	50	
	6	9	350	300	
	7[c]	10	1450	250	
	8	18	1300	10	
	9	5	1050	750	
	10	19	1050	200	
	11	10	400	150	
	12[c]	3	1150	400	FECR: 88%
	13	8	250	10	LC: 51%
Mean		10	846	190	R/S: R
May 2004	1	19	300	50	
	2	20	600	50	
	3	6	1450	250	
	4	4	450	0	
	5	10	1200	100	
	6	11	750	50	
	7	22	50	200	
	8	11	100	100	FECR: 89%
	9	9	50	0	LC: 43%
Mean		12	550	89	R/S: R

[a]LC, lower 95% confidence limit; R, resistance; S, susceptibility
[b]Dewormed with ivermectin on day 7
[c]Dewormed again with pyrantel on day 7

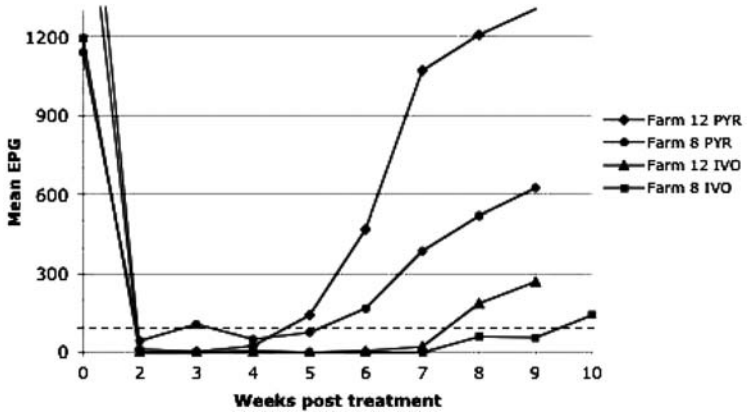

Figure 1. Results of faecal egg counts on two farms following treatment with either pyrantel (Pyr) or ivermectin (Ivo) on day 0. Egg reappearance period (ERP) was defined as the number of weeks before the mean figure for strongyle eggs per gram of faeces had exceeded 100

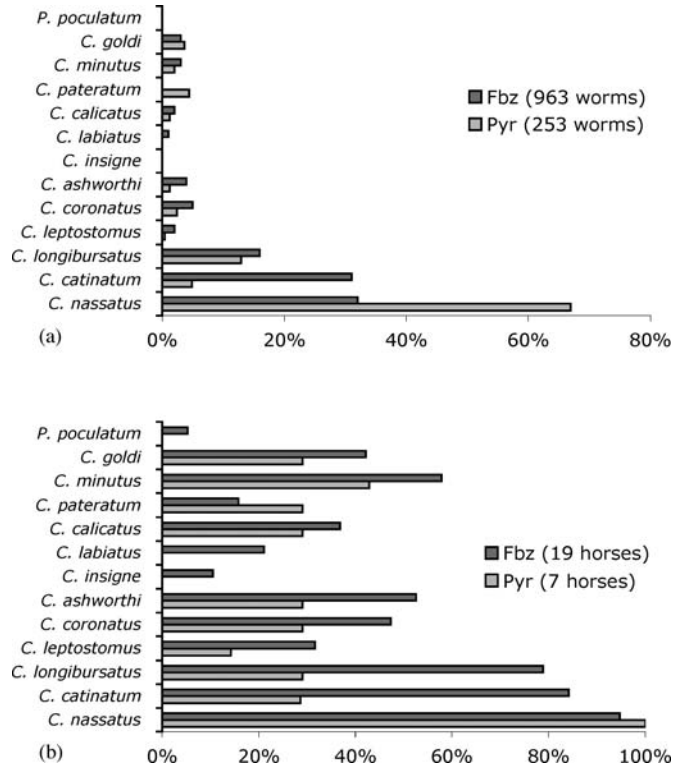

Figure 2. (a) Frequency and (b) prevalence of adult cyathostomins expelled from 19 fenbendazole (Fbz)-treated and 7 pyrantel (Pyr)-treated horses that still excreted at least 150 epg after 14 days

similar to that observed in Sweden a decade ago (Osterman *et al*., 1996), which indicates that the efficacy of this drug is still acceptable. Nevertheless, FECRT performed on two farms where the effect of pyrantel was found to be equivocal in April–May 2000 revealed the existence of pyrantel resistance on one of these farms when it was re-investigated two years later. In sheep it has been shown that the result of FECRT may vary in the same flock over the year (Anderson *et al*., 1988), and it is therefore important to critically assess the outcome of this test. However, on the pyrantel-resistant farm in the present study the same results were obtained when the test was performed for three consecutive years.

The efficacy of fenbendazole was unsatisfactory; more than 70% of the farms met the criteria for resistance 14 days after deworming and the mean FECR was 86%. The proportion of benzimidazole-resistant farms was in accordance with reports of anthelmintic resistance in horse establishments throughout Europe (Norway, Ihler (1995); Denmark, Craven *et al*. (1998); Slovakia, Várady *et al*. (2000)).

The use of (pro)benzimidazoles in horses in Sweden has been very low for the last 15 years. Since 1994 these anthelmintics have accounted for less than 5% of the market (Osterman Lind *et al*., 2005). It was therefore interesting to compare the results from the present study with those obtained in 1986 (Nilsson *et al*., 1989). The appearance of a higher mean FECR (86% vs 67%) and a lower proportion of resistant farms (56% (recalcuted according to Nilsson *et al*. (1989)) vs 96%) in the present study was not statistically significant ($p = 0.2$). Thus, it could not be concluded that reversion to susceptibility occurred in this investigation in Sweden, although this phenomenon has been suggested to occur in a recent study in the UK (Jones *et al*., 2003). In another study, including three farms, reversion did not occur when horses were withdrawn from treatment with benzimidazoles for 2–3 years (Uhlinger and Johnstone, 1984).

It has been clearly demonstrated that the classification of farms as resistant or not is dependent on the choice of FECRT method (Craven *et al*., 1998; Cabaret and Berrag, 2004). Over the years, numerous ways of calculating and interpreting the status of anthelmintic resistance by FECRT have been suggested, but the method has not yet been standardized for horses (Kaplan, 2002). In the present study, untreated control groups were not included, since some farms had too few egg-positive horses. For this reason, and because of great individual variations in FECR within the treatment groups, we chose the protocol of Bjørn and colleagues (1991). This method is based on geometric means of epg and does not include groups with untreated control horses. The advantage of using geometric means is that the impact of individuals with extreme values is diminished.

It has been suggested that resistance criteria should be based on original drug efficacy data (Pook *et al.*, 2002). In accordance with that suggestion, the FECR cut-off value of 95% was set for fenbendazole and ivermectin, whose efficacies against susceptible cyathostomins were reported to be >95% (Klei and Torbert, 1980; Malan *et al*., 1981). For pyrantel, however, initial studies showed efficacies of the order of 90–92% (Cornwell and Jones, 1968; Lyons *et al*., 1974), which implies that FECR values of 90–95% may very well occur in susceptible treatment groups. To increase the specificity of the test, we chose the FECR level of 90% for pyrantel. If the 95% cut-off had been used also for pyrantel, farm no. 3 would have been assessed as pyrantel resistant**.**

It is generally recommended that the post-treatment FECs should be performed 10–14 days following anthelmintic treatment (Coles *et al*., 1992). In this study we also were

interested to see whether the number of days elapsing from treatment until faecal sampling was important for the assessments. The mean FECR values for ivermectin and pyrantel were 99–100% on all three sampling days, whereas for fenbendazole the FECR was significantly higher on day 7 than on days 14 and 21 post treatment. Eight farms in the fenbendazole group converted from suspected resistant on day 7 to resistant on day 14. The most plausible explanation is that the egg production of resistant female parasites was temporarily suppressed by the anthelmintic treatment. This has been observed for *Ostertagia* spp. in sheep (Martin *et al*., 1985). Another possible explanation could be that resistant female cyathostomins that survived the anthelmintic treatment compensated for the loss of susceptible worms.

The cyathostomins expelled and identified following the second anthelmintic treatment were obviously adults or late larval stages that survived the first treatment with fenbendazole or pyrantel. However, it cannot be excluded that they had developed from encysted larval stages between the two anthelmintic treatments. Therefore we present the data only on the adult worms that were expelled, because these would have needed more than 21 days to develop from larval stages. The frequencies of expelled, species that were presumably resistant were approximately the same for fenbendazole and pyrantel, with the exceptions of *C. nassatus* and *C. catinatum*. Of the 13 species identified from horses initially dewormed with fenbendazole, 11 have been reported to be benzimidazole resistant (Kaplan, 2002). They are also the most prevalent species in cyathostomin populations. From horses initially treated with pyrantel, 10 species were identified; *C. nassatus* was the major species found, comprising 68% of the recovered worms. Chapman and colleagues (1996) noted a reduced efficacy of pyrantel against seven cyathostomins. These species, except for *Coronocyclus labiatus,* were also recovered in this study.

In conclusion, this study has shown that benzimidazole- and pyrantel-resistant populations of cyathostomins are present in Swedish horse establishments. Although the efficacy of pyrantel was still acceptable on the majority of the farms, the emergence of resistance against two of the three anthelmintic classes used in horses implies concern for the future of anthelmintic chemotherapy. It is recommended that FECRT should be used regularly to monitor the resistance status on stud farms. For accurate assessments, more than 7 days should lapse from deworming until faecal sampling, calculations should be based on geometric means, and treatment groups should consist of at least 6–8 horses.

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