A field study to evaluate the efficacy of fenbendazole on 9 stud farms

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ABSTRACT: A study was conducted to determine the efficacy of a benzimidazole anthelmintic (fenbendazole) on horse farms in Slovakia. Nine stud farms with a total number of 80 horses were selected for the faecal egg count reduction (FECR) test. Resistance was assumed if egg count reduction was less than 90%. A low level of benzimidazole resistance was found on three farms (84.4–89.0%). On the remaining farms the results of the FECR test indicated substantial reduction in faecal egg output after treatment (92.5–99.4%). Larval identification before treatment revealed *Cyathostominea* larvae as being predominant.

Keywords: horses; fenbendazole; cyathostomins; resistance

Anthelmintic resistance (AR) is a common cause of failure in worm control programs. The increasing occurrence of AR in cyathostomins (Lichtenfels *et al*., 2002) is an important veterinary and animal welfare problem, as cyathostomins constitute the most common group of internal parasites of horses. Three main classes of anthelmintics are used to control cyathostomins: benzimidazoles, tetrahydropyrimidines, and macrocyclic lactones. All were effective when they were first introduced, however, resistance to benzimidazoles has been widely reported (Conder and Campbell, 1995; Kaplan, 2002) and resistance to the tetrahydropyrimidine pyrantel has been recorded in USA (Chapman *et al*., 1996; Lyons *et al*., 2001; Tarigo-Martinie *et al*., 2001) and Europe (Craven *et al*., 1998). To date, there have been no reports of resistance to macrocyclic lactones ivermectin or moxidectin.

Resistance of cyathostomins to benzimidazole anthelmintics in Slovakia has recently been recorded on 14 out of 19 farms, with efficacy values ranging from 65.1 to 86.3% (Varady *et al*., 2000). The purpose of this study was to continue the evaluation of the efficacy of benzimidazole anthelmintics on stud farms, with special emphasis on the occurrence of resistant cyathostomins on these farms.

MATERIAL AND METHODS

The survey was conducted between September 2000 and June 2001 on 9 stud farms, including a total number of 80 horses. The farms were selected randomly and the only requirements were that a minimum of 10 horses be available for examination of faecal samples on each farm and that the animals had not been treated with an anthelmintic for at least 10 weeks prior to the study. On each farm horses with the highest faecal egg counts received fenbendazole at 7.5 mg/kg body weight (BW) administered orally in the paste formulation (Panacur®, Intervet). A minimum of six horses per farm was used to calculate FECR. To calculate the dose of each drug, the BW of each horse was estimated by calibrated tape measurement of the heart girth. Faecal samples were collected from selected horses and stored at 5°C in a cooling box until laboratory examination. A modified McMaster

Supported by the Scientific Agency of the Slovak Academy of Sciences – VEGA (Grant No. 2/4180/04).

technique (Coles *et al.*, 1992) with a sensitivity of 15 eggs per gram (epg) was used for the detection of strongyle eggs in faecal samples. One week after the first visit, the farm was visited again and faecal samples were obtained only from those animals that had shown a positive epg on the first occasion. Immediately after faecal sampling during a second visit, the horses were treated with anthelmintic according to label instructions. The farms were visited again within 10–14 days after treatment and the treated animals were again sampled. Larval cultures (pre- and post-treatment) were made from pooled samples according to the method of Roberts and O'Sullivan (1950) using about 10 g faeces per animal, and incubated at 27°C for 12–14 days. The third stage larvae were harvested and differentiated according to MAFF (1986).

Since no control group was used in this study FECRs were calculated for each horse individually using formula:

$$[(\text{pretreatment FEC} - \text{posttreatment FEC}/\text{pretreatment FEC}] \times 100$$

and mean reduction for the treatment group for each farm was calculated. According to the recommendations of the World Association for the Advancement of Veterinary Parasitology (WAAVP) for detection of AR in horses (Coles *et al.*, 1992), helminths are considered resistant when the FECR using arithmetic means, is <90%.

RESULTS

Five of the 14 farms examined were excluded because too many horses showed faecal egg counts lower than 15 epg and thus a treatment group with a minimum of six horses could not be established. Larval identification before treatment revealed *Cyathostominea* larvae as being predominant. In the larval cultures from farms No. 1, 5, 6 and 7 only *Cyathostominea* larvae were present. On farm 3, the composition was as follows: *Cyathostominea* 95%, *Strongylus vulgaris* 3% and *Trichostrongylus axei* 2%. Larval cultures from farm 4, 8 and 9 consisted of two species: *Cyathostominea* (>98%) and *Gyalocephalus capitatus* (<2%). Post-treatment cultures consisted solely of *Cyathostominea* larvae. The efficacy of fenbendazole based on arithmetic mean of egg counts of each horse before and after treatment is shown in Tables 1, 2 and 3. A total of 80 horses from 9 farms were treated with fenbendazole and per-farm FECRs ranged from 84.4 to 99.4%. Given the criteria that resistance is indicated if the FECR is <90%, BZ resistance was detected on 3 of the 9 farms examined. The ovoscopical findings of

Table 1. Effect of fenbendazol – 7.5 mg/kg BW on faecal egg counts (epg) on horses from farms 1–3

Animal No.	Farm 1			Farm 2			Farm 3		
	Day 0[a]	Day[b]	FECR[c]	Day 0[a]	Day 10[b]	FECR[c]	Day 0[a]	Day 10[b]	FECR[c]
1	165	0	100	315	0	100	255	0	100
2	180	0	100	165	0	100	75	0	100
3	315	0	100	150	15	90.0	45	0	100
4	510	30	94.1	270	0	100	210	30	85.7
5	240	0	100	225	30	86.6	60	15	69.2
6	105	0	100	90	15	83.3	90	0	100
7	345	0	100	30	0	100			
8	90	0	100	30	30	0			
9	180	0	100	30	0	100			
10	360	0	100						
FECR[d] (%)			99.4			84.4			92.5

[a]EPG before treatment; [b]EPG 10–14 days after treatment; [c]individual faecal egg count reduction; [d]faecal egg count reduction for the farm

Table 2. Effect of fenbendazol – 7.5 mg/kg BW on faecal egg counts (epg) on horses from farms 4–6

Animal No.	Farm 4			Farm 5			Farm 6		
	Day 0[a]	Day [b]	FECR[c]	Day 0[a]	Day [b]	FECR[c]	Day 0 [a]	Day [b]	FECR[c]
1	90	0	100	195	0	100	75	15	80.0
2	150	0	100	150	0	100	105	60	42.8
3	180	0	100	210	0	100	270	30	88.8
4	150	0	100	180	0	100	225	0	100
5	195	15	92.3	150	0	100	255	0	100
6	75	0	100	180	0	100	165	0	100
7	300	0	100	135	0	100	225	0	100
8	180	0	100	270	15	94.4	15	0	100
9	120	0	100	135	0	100			
10	90	0	100	300	0	100			
FECR[d] (%)			99.2			99.4			89.0

[a]EPG before treatment; [b]EPG 10–14 days after treatment; [c]individual faecal egg count reduction; [d]faecal egg count reduction for the farm

Table 3. Effect of fenbendazol – 7.5 mg/kg BW on faecal egg counts (epg) on horses from farms 7–9

Animal No.	Farm 7			Farm 8			Farm 9		
	Day 0[a]	Day[b]	FECR[c]	Day 0[a]	Day [b]	FECR[c]	Day 0[a]	Day[b]	FECR[c]
1	45	15	66.6	270	15	94.4	435	0	100
2	405	75	81.5	210	60	71.4	60	0	100
3	585	75	87.2	180	30	83.3	105	15	85.7
4	480	60	87.5	75	0	100	180	0	100
5	90	15	83.3	90	0	100	60	15	75.0
6	75	0	100	255	0	100	405	30	96.2
7	135	15	88.8	345	0	100	285	0	100
8	330	75	77.2				90	0	100
9	30	0	100						
10	90	15	83.3						
11	60	0	100						
12	60	0	100						
FECR[d] (%)			87.9			92.7			94.6

[a]EPG before treatment; [b]EPG 10–14 days after treatment; [c]individual faecal egg count reduction; [d]faecal egg count reduction for the farm

faecal samples of the horses from the farm declared as sensitive (farm No. 1, 3, 4, 5, 8 and 9) demonstrated that none or maximum two horses per farm have FECR below 90%. On the other hand three to eight horses from the farms declared as resistant had egg reduction lower then 90%. In addition to the above, ten animals out of 80 were also positive (15–90 epg) for *Parascaris equorum*, and treatment with fenbendazole reduced the egg counts to negative values for each horse.

DISCUSSION

The results of the faecal egg count reduction test indicated that benzimidazole resistance of small strongyles (*Cyathostominea*) was present on three farms. In Slovakia, an earlier report (Varady and Corba, 1997) revealed the presence of BZ resistant cyathostomins on two selected stud farms. Together with the results of a survey performed in 1998 and 1999 (Varady *et al.*, 2000) the possibility of widespread AR of horse strongyles in Slovakia was indicated. The results of the present investigation, however, do not confirm this.

In the previous survey 1999 (Varady *et al.*, 2000) resistance to benzimidazoles was detected on 14 out of 19 farms, with FECR values indicating resistance ranging from 65.1 to 86.3%. On 10 of these farms, the efficacy of anthelmintic treatment with benzimidazoles ranged from 70–90%. This would indicate a low level of resistance on these farms. In the present study, a low level of resistance was detected on only three farms, which is surprisingly lower, compare to the results of the previous survey. The small questionnaire study performed during survey showed similar parasite control in all of the stables. The horses were treated 2–3 times per year during a period of last 5 years. In all stables, with exception of farm 4 and 5 an alteration of fenbendazole and macrocyclic lactone drugs was used. The most important factors in choosing an anthelmintic were the possibilities of direct administration, the experience of 'good effect' of the anthelmintic and the veterinarian's recommendation. However, none of the herd owners performed faecal egg counts before treatment. Majority of horses included in this study came from racing studs where horses are moved between farms quite frequently, mixing of different worm populations of small strongyles is likely. Another explanation might be that most farmers use an annual rotation program for anthelmintics, where benzimidazole products are substituted with macrocyclic lactones. Under such conditions the rate of selection for anthelmintic resistance in small horse strongyles would be reduced.

Annual rotation (slow) between different classes of anthelmintics is considered by some as an appropriate method to avoid or delay development of AR in parasites (Coles and Roush, 1992). On farms with a history of BZ resistance, an annual rotation of tetrahydropyrimidines and macrocyclic lactones might be adopted in an attempt to minimize the rate of selection for AR. Although slow rotation is generally accepted as the best approach for delaying resistance, most effective approach is to treat simultaneously with two chemically distinct anthelmintics (Kaplan, 2002).

In order to reduce the risk of transmission of resistant cyathostomins from one farm to another, horses must be treated with an effective anthelmintic before being introduced to a new farm. However the most available drug treatments do not kill the mucosal larval stages, which are much more numerous than the lumenal adults. For this reason five daily treatment with fenbendazole (Duncan *et al.*, 1998; DiPietro e*t al.*, 1997) should be applied upon arrival to remove encysted mucosal larvae.

The early detection of reduced effectiveness is one of the important factors in delaying AR on horse farms. The faecal egg count reduction test is the most frequently method to detect resistance in the field. *In vitro* testing, using either an egg hatch assay (Coles *et al.*, 1992) or larval development (Ihler and Bjørn, 1996) tests have been used in detecting of AR in small strongyles. Unfortunately, both methods have some limitations. Low sensitivity and poor correlation with *in vivo* results have been cited in the evaluation of benzimidazole resistance in cyathostomins (Craven *et al.*, 1999; Fisher *et al.*, 2001; Tandon and Kaplan, 2002). In the future molecular techniques might offer some advantages with high specificity and sensitivity, and a PCR diagnostic assay (von Samson-Himmelstjerna *et al.*, 2002) may prove a useful tool for the assesment of allele frequencies in benzimidazole sensitive and resistant populations of small strongyles.

Based on the results of the present study as well as previous prevalence studies in Slovakia, testing for efficacy of anthelmintic drugs should be performed regularly (every 2nd year) to ensure effective control of small strongyle in horses.

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Received: 03–08–18
Accepted after corrections: 04–01–19

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