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SURVEY ON THE ANTHELMINTIC EFFICACY OF BENZIMIDAZOLES AGAINST GASTROINTESTINAL STRONGYLIDS OF SHEEP IN SOUTHEASTERN BULGARIA

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ABSTRACT

The efficacy of benzimidazole anthelmintics was investigated in 15 randomly selected sheep farms located in southeastern part of Bulgaria. The *in vitro* egg hatch assay (EHA) was performed after anaerobical storage of mixed faecal samples from each farm for 2 – 5 days. Pure thiabendazole was used in solutions of 0.02; 0.04; 0.05; 0.08 and 0.1 $\mu g.ml^{-1}$. Effective dose (ED_{50}) was calculated. On each farm, 30 sheep were allocated at random into two groups of 15 animals each: an untreated control group and a group that was orally administrated albendazole (Valbazen®, Pfizer Animal Health) (3.8 $mg.kg^{-1}$) or oxfendazole (Oxfenil®, Virbac Animal Health) (5.0 $mg.kg^{-1}$). Individual faecal egg counts were estimated before and 14 days after treatment and faecal egg count reduction (FECR) was calculated, for performing the *in vivo* egg faecal count reduction test (FECRT). Mixed farm faecal samples were cultivated for 7 days at 27^{o}C for developing and identification of strongylid larvae III. The predominant genus was *Haemonchus*.
In all the examined sheep farms the ED_{50} of thiabendazole was below 0.1. All the flocks showed FECR values over 95%. No developed anthelmintic resistance was detected. From the results of the present trial can be concluded that benzimidasoles are high effective against sheep gastrointestinal strongylids in southeastern Bulgaria.

Key words: benzimidazoles, efficacy, resistance, sheep, strongylids.

INTRODUCTION

Sheep are very important farm animals for the animal husbandry in Bulgaria. According to the official report of the Ministry of Agriculture and Forests from 2003 the approximate number of the sheep in the country was 2.5 million. Most of the breeding stocks are kept under extensive conditions, in small private farms which are distributed all over the country. The home trade, as well as the traffic of the animals from farm to farm are not well controlled by the governmental institutions. Thus, there is a risk drug-resistant populations of parasites to be introduced into the flocks.

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In the past, levamizole was the most common anthelmintic substance for controlling round worm infestations in sheep in Bulgaria. In 80s began the mass use of benzimidazoles against gastrointestinal strongylids of sheep. Very often the farmers treat the animals without any parasitological investigations, recommendations and control on behalf of veterinarians. The indiscriminate use of the drugs, in many cases without considering the body weight of the heaviest animal in the group, increases the risk of developing anthelmintic resistance strains among gastrointestinal nematodes in sheep.
The development of resistance to anthelmintic drugs is a major threat to parasite control world-wide (1). Benzimidazole, levamisole/morantel and ivermectin resistances occur in nematodes of sheep (2).
Benzimidazole resistance in sheep gastrointestinal strogylids has been reported in many European countries as Germany,

Austria, Sweden, Denmark, Netherlands, Belgium, France, UK, Ireland, Czech Republic, Slovak Republic, Italy (for review see 3) including Greece (4) and Turkey (5). As Greece and Turkey are neighbor countries of Bulgaria, it is possible resistant sheep nematode strains to be imported in our country.

The aim of the present study was to estimate the anthelmintic efficacy of two benzimidazoles against gastrointestinal strongylids of sheep in southeastern Bulgaria, as well as to check the presence of developed resistance to these drugs.

MATERIALS AND METHODS

Farms and animals. During the period from February 2002 to June 2005 15 sheep farms located in southeastern part of Bulgaria, where the sheep breeding is an important livestock brunch, were randomly selected and examined. All of them belonged to private owners. Sheep were kept under semi-intensive or extensive pasture breeding. The farms consisted mainly of the local Stara Zagora and Black-head Pleven breeds or crosses. The number of animals in each farm varied between 50 and 170 and their age was from 12 to 24 months. All the animals were identified by ear tags. They showed satisfactory clinical conditions and were not dewormed during last 10 weeks before the trial.

All the farms were visited a week before the trial to check the egg output. Faecal samples from 15-20 selected at random animals were examined by the modified McMaster technique (6) to estimate the mean EPG (faecal egg count). Only flocks with mean EPG higher than 150 were included in the experiment.

Grouping, sample collection and processing. On each farm 30 randomly selected sheep were used for performing the assays. They were divided in two groups: (i) Group T – treated (n = 15) and (ii) Group C – untreated controls (n =15). On day 0 individual faecal samples in quantities of approximately 20 g were collected from the rectum of all the experimental sheep. The materials were transported in a cool box to the laboratory where all the samples were divided into 3 parts: (i) 3 g used for in *vivo test*; (ii) about 2 g from each sample were used for preparing a mixed sample for *in vitro* test; (iii) the remains of the individual samples were homogenized and cultivated for 7 days at $27^{o}C$ for developing strongylid larvae III. The larvae were collected using the routine Baermann technique and were stored at $2-4^{o}C$ until their identification.

On day 14 after treatment individual faecal samples from all the trial animals were taken for performing the *in vivo* test.

Egg hatch assay (EHA). After collecting, a mixed flock sample in approximate volume of 50-60 g was homogenized in a plastic jar with tap water and stored anaerobically at room temperature for 2 – 5 days. The *in vitro* test was performed as described by Coles et al. (6). Pure thiabendazole was used in solutions of 0.02; 0.04; 0.05; 0.08 and 0.1 $\mu g.ml^{-1}$. Effective dose (ED_{50}) was calculated using a regression analysis.

Faecal egg count reduction test (FECRD). For performing the *in vivo* test individual faecal samples from both the treated and control groups were examined twice – on day 0 (before treatment) and on day 14 (after treatment) following the description (6). A modified McMaster technique with a sensitivity of 50 eggs per gram of faeces was used for faecal egg counting. The mean faecal egg count in the treated group (EPG_t) in each farm was estimated and compared to the mean egg count for the respective control group (EPG_c). The percentage of faecal egg count reduction ($FECR_{\%}$) was calculated using the formula : $FECR_{\%} = 100 \times (1 - EPG_t/EPG_c)$.

Drugs and treatment. All the sheep from groups "T" were treated on day 0 parallel with the collecting faecal samples. One of the anthelmintics namely albendazole (Valbazen®, Pfizer Animal Health) (3.8 $mg.kg^{-1}$) or oxfendazole (Oxfenil®, Virbac Animal Health) (5.0 $mg.kg^{-1}$) was used in the different farms. Doses were fixed according the heaviest animal in the group. The drugs were orally administrated using calibrated syringes.

Table 1 shows the primary data about the farms, level of infection before the trial, and the treatment.

RESULTS

The faecal cultures indicated that *Haemonchus* was the most common genus of sheep gastrointestianal strongylids. It was found in all the 15 examined farms with a quota of 2 - 71%. Other genera recorded were *Ostertagia* – in 14 farms (0 – 62%); *Trichostrongylus* – in 14 farms (0 – 57%); *Chabertia* / *Oesophagostomum* – in 13 farms (0 – 44%); *Cooperia* – in 9 farms (0 – 37%) and *Bunostomum* – in 4 farms (0 – 21%).

Faecal egg counts, faecal egg count reductions and the results of egg hatch assay for benzimidazoles are presented in table 2. In all but one examined sheep farms the FECR was 100%. Only in farm no. 12 the FECR was 98.4. The ED_{50} values ranged from 0.002 to 0.041.

As all the percentages of FECR are over 95%, and all the values of ED_{50} were below 0.1, these results indicated that the bezimidazoles possess high level of efficacy against gastrointestinal nematodes of sheep, and also the examined worms have not developed resistance to benzimidazole anthelmintics in southeastern part of Bulgaria up to the time of the present trial.

DISCUSSION

According to a definition: “Resistance is present when there is a greater frequency of individuals within a population able to tolerate doses of a compound than in a normal population of the same species and that is heritable” (2).

The data obtained in our studies showed that strongylid strains in studied sheep farms were sensitive to benzimidazoles, and that, according to the cited definition excludes the existence of anthelmintic resistance.

Earlier studies in some sheep farms in West Bulgaria proved neither a developed anthelmintic resistance to benzimidazoles in gastrointestinal strongylids (7). In some herds however, the authors detected anthelmintic resistance against levamisole. This result could be explained by the fact that levamisole is an older and yet, more commonly used preparation in Bulgaria compared to benzimidazoles. The probable reason for this is the availability of a local levamisole brand on the market, which makes it less expensive and preferred by owners.

Provided that in our country there are conditions determining the selection of anthelmintic resistance, we could hypothesize that the extensive application of benzimidazoles in the veterinary practice could result in the appearance of resistance against them. Thus, Papadopoulos et al. (8) did not detect anthelmintic resistance against benzimidazoles in trichostrongylids on sheep in Greece, but only a year after, the same author already reported the existence of this event (9).

The identical results from the application of both FECRT and EHA tests showed that they are equally suitable for evaluation of the sensitivity of gastrointestinal strongylids of sheep to anthelmintics from the benzimidazole group. Similar results in a simultaneous use of the same two tests are reported in India (10).

The absence of anthelmintic resistance in gastrointestinal strongylids of sheep to benzimidazoles reported by us and other authors (7) should not sound encouraging. While it is still possible, with regard to the global scale of the problem, the attention of parasitologists, pharmacists, veterinary specialists and farmers in Bulgaria should be focused on the performance of urgent measures and the invention of proper strategies for prevention of the selection and import of anthelmintic resistance in Bulgaria.

Table 1. *Number and body weight of the sheep, mean EPG before the trial and doses of anthelmintics used*

Farm no.	Total no. of sheep	Body weight (kg)	Mean EPG before trial	albendazole (mg)	oxfendazole (mg)
1	150	50-60	400	228	-
2	50	50-60	255	228	-
3	60	60-70	300	266	-
4	70	60-70	310	266	-
5	50	60-70	450	266	-
6	150	50-60	420	-	317
7	60	50-60	460	-	317
8	50	50-60	180	-	317
9	90	40-50	170	-	272
10	170	60-70	160	-	363
11	150	60-70	170	-	363
12	130	40-50	1 030	-	272
13	60	60-70	390	-	363
14	100	50-60	210	-	317
15	55	40-50	190	-	272

Table 2. *Mean faecal egg counts (EPG), percentages of faecal egg count reduction (FECR), lower limits of the 95% Confidence Interval after treatment and egg hatch assay (EHA) ED_{50} in 15 sheep farms in Bulgaria*

Farm no.	Control	Treated				
	EPG	EPG before treatment	EPG after treatment	FECR (%)	95% CI Lower limit	EHA ED_{50}
1	500	300	0	100	100	0.004
2	180	310	0	100	100	0.005
3	230	330	0	100	100	0.016
4	330	250	0	100	100	0.005
5	440	460	0	100	100	0.011
6	280	450	0	100	100	0.013
7	200	520	0	100	100	0.009
8	170	190	0	100	100	0.015
9	160	170	0	100	100	0.013
10	170	210	0	100	100	0.016
11	150	200	0	100	100	0.015
12	1 080	640	10	98.4	90.9	0.041
13	220	550	0	100	100	0.010
14	220	420	0	100	100	0.002
15	340	240	0	100	100	0.032

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