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# Evaluation of closantel and mebendazole for the reduction of faecal egg count in sheep nematodes

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The type and the level of invasion by the endohelminths from nematodes and trematodes species were measured in naturally infected sheep by counting the number of eggs in faeces (FEC). The samples of faeces were taken before the spring grazing from randomly selected experimental group of sheep 7 days prior to (Day-7) and on the day of medication (Day 0, n = 15). Closantel (10 mg $kg^{-1}$ body mass) and mebendazole (15 mg $kg^{-1}$ body mass) were applied orally. On the $7^{th}$ and $21^{st}$ day after the treatment the value FECp was verified in the same group of animals and the degree of reduction with respect to the basal value FECb on the Day-7 and Day 0 (FECRT; 100 x (1-(FECp/FECb)) was calculated. All species of endohelminths (*Ostertagia, Trichostrongylus, Nematodirus, Strongyloides, Toxocara, Moniezia, Chabertia and Trichuris*) found reacted to application of the drug by intensive reduction of the count of eggs excreted, in the range from 94% to 100%.

Key words: sheep, nematodes, anthelminitic, faecal egg count reduction test

## INTRODUCTION

Prevention and treatment of parasitic infections is one of key conditions for successful intensive breeding of small ruminants, usually raised on pastures. Negative effects of parasites on the host's organism are outstanding in reduced consumption of dry matter of the feed, in the efficiency of energy utilisation of the feed and reduction of the level of total blood proteins, particularly albumins. All this results in reduced growth of lambs being fattened, lower milk production and growth of the fleece of adult sheep (Sykes 1994). During the last decade the resistance of gastrointestinal nematodes to endoantiparasitics in sheep has been continuously increasing. It is estimated (Williams 1997) that today's highest degree of resistance is noted in the Southern hemisphere, i. e., in Australia, Africa and Latin America. The first report at all on the resistance of sheep nematodes to anthelminthic drugs has come from the U.S.A. (Drudge et al. 1957). Due to strict control of ways of distribution and use of veterinary drugs in Europe the resistance occurred later, namely, the first time in Switzerland (Jordi 1980), then in Great Britain (Britt 1982), Netherlands (Boersema et al. 1982), France (Kerboeuf and Hubert 1985) and Germany (Duewel et al. 1987).

In general, the term resistance is understood to be the characteristic of the parasite to withstand such high doses of the drug that would kill the majority of subjects in the population of parasites of the same species. The parasite can acquire such nature by mutations or by gradual phenotypic expression of existing genes as a result of the adaptive capacity to changes in the environment. Thus, the resistance of the hematophagous nematode *Haemonchus contortus* to avermectin is related to one gene only and, consequently, spreads much faster than if it were polygenous (Jackson and Coop 2000).

The factors influencing the intensity of selection of resistant nematodes are primarily related to the individual properties of the host animal, type of breeding, portion of the parasitic population in *refugia* (which is outside the host animal on the pasture) during the period of treatment, frequency of application of the anthelminthic and degree of underdosing. The latter is considered to be the most frequent mistake of the breeders in practice, since, as a rule, the breeders give the anthelmintics in relation to the average body weight of the sheep in the flock, which has a significant influence on the survival of parasites and help the resistant alleles in the population to cumulate. The verification of resistance to anthelmintics is conducted in order to evaluate first, the efficiency of the individual drug and in the second place, the general strategy of anthelmintics use. There are several methods of evaluation of anthelmintic resistance. In the field practice *in vivo* method of faecal egg count reduction test (FECRT) is widely applicable.

This research will study whether the resistance of gastrointestinal nematodes to widely used anthelmintics closantel and mebendazole occurs in the naturally infected flock. The results may be of help in projecting the general strategy of prevention of the parasitic infections, since there are no data at all on the resistance of nematodes to endoantiparasitics in Slovenia.

## MATERIAL AND METHODS

The experiment was carried out on a naturally infected flock of 160 sheep of Jezersko-Solčava breed. The entire flock grazes on the pasture from May to late October. The average body mass of grazing animals was 40 kg. The age of grazing sheep was between one to five years. The Jezersko-

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Solčava sheep are naturally polyoestric, therefore, they lamb throughout the entire grazing season.

The pasture is located at 420 m a.s.l., receives 1.400 mm of precipitation and 250 hours of insolation in year before the experiment was performed. The entire grazing area was divided into three grazing sections: one larger section (4 ha) and two smaller sections (1.3 and 1.0 ha). In the larger section the sheep are grazing 30 days; then they move to the smaller section for 10 days and, finally, into the smallest section, where they are grazing 8 days. The average stocking density expressed in live mass of animals was 1000 kg $ha^{-1}$ of grazing area.

In the botanic composition of the herbage the grasses are predominant: meadow fescue (*Festuca pratensis*), red fescue (*Festuca rubra*), sheep's fescue (*Festuca ovina*), cocksfoot grass (*Dactilys glomerata*), white clover (*Trifolium repens*), red clover (*Trifolium pratense*) and Italian ryegrass (*Lolium multiflorum*). The sheep grazed together with the lambs. Beside of fresh harbage sheep received the feed concentrate after return into the stable in the evening. The mineral-vitamin mixture and water were available to animals only in the stable.

Seven days before the application of the anthelminthic (Day-7; n = 7) and on the day of treatment (Day 0, n = 15) the type and level of infection were determinated. In the samples of faeces of experimental animals the genus and species of the endoparasite were determined by the flotation (saturated NaCl solution) and sedimentation methods for the preliminary evaluation and in order to have a general impression into the parasitic population. The third sampling of faeces was carried out seven days later (Day 7; n = 15) and the fourth sampling 21 days after treatment (Day 21). The test was carried out at the end of March, when the animals were still in the stable. Using the drench gun the emulsion[1] of closantel (50 mg $mL^{-1}$) and mebendazole (75 mg $mL^{-1}$) was applied orally to 15 randomly selected sheep on the body mass basis in amount of 10 mg of closantel $kg^{-1}$ and 15 mg $kg^{-1}$ of mebendazole.

The individual samples of faeces from all sampling of the same animals were investigated quantitatively with respect to the number of eggs in faeces. The method of counting in a small glass chamber was used first by Gordon and Whitlock in year 1939 and described by Whitlock (1948) in the McMaster laboratory in Sydney, Australia. So far, the original method has been subjected to several modifications; mostly, they refer to the degree of faeces dilution before counting. In this experiment detailed technique recommended by the World Association for the Advancement of Veterinary Parasitology (WAAVP) described by Coles et al. (1992) was taken into consideration[2]. As the result, the lower detection cut off was at 25 worm eggs per g of faeces. The degree of faecal egg count reduction in 1 g of faeces for the particularly species of nematodes (FECR) was tested according to the modified standard WAAVP formula (FECR = 100 × (1-(FECb/FECp)), where FECb is expresed as the arithmetical mean of the egg count in $g^{-1}$ of faeces before treatment and FECp after the treatment of the same group of animals. This approach, differing from the original method in the test on repeated sample which produce an autocorrelation inside the test groups, is similar to the methodology according to Bjorn et al. (1991), but it retains original algebraic operations.

Our statistical approach does not know the independent control group of animals; therefore, instead of the confidence interval for FECR (used by WAAVP method) the confidence interval in the population for the average count FEC on the test day 21 was calculated. For the lower and upper limit of that interval the expected degree of the egg count reduction at 95% confidence level was estimated.

In addition, the hypothesis that the proportions of infected animals with respect to the number of all examined animals before and after treatment does not differ significantly was tested. The proportions were tested by the non-parametric method for the group of dependent samples according to McNemar test with known computer algorithm[3].

## RESULTS AND DISCUSSION

The average values of the egg count of the particularly parasites in 1 g of faeces in the observed periods are presented in table 1. Taking into account the criteria (Abbott and Maxwell 2002) associating the number of the eggs excreted and the nematodes worm burden in forstomachs, stomachs and/or intestines it can be supposed that the infestation was very strong on the Day -7, although no clinical symptoms could be observed during inspection of the flock. Seven days after the first coprological examination, i.e. on the day of application of the anthelmintic (Day 0), the faeces samples were even negative for the presence of the eggs of Toxocara, although the animals were infected.

Seven days after application the first effect of the dehelminthization was established. Comparison of proportions of the infected and healthy animals before and after treatment shows that the portion of invaded sheep was reduced significantly ($Chi^2 = 70.936$, df = 1, $p<0.0001$). It is obvious that the therapeutic combination of closantel and mebendazole is appropriate for treating nematode and some trematode infections. However, it is questionable whether the efficiency of this combination is corresponding to the particularly species of helminths. Only in case of nematodes genera Ostertagia and Trichstrongylus, wich colonize the forestomachs, the terapeutic effect, measured by the percentage of FEC reduction on the Day 7, was perfect and immediate. The portion of reduction for the genera Strongyloides is somewhat lower (98%), but the average egg count in g-1 of faeces on the Day 7 is greater than for the genus Ostertagia. It can be expected for both mentioned genera with high probability (95%) that faecal excretion will not exceed more than 13 and 11 eggs in $g^{-1}$ of faeces, respectively (see Table 1).

According to the general knowledge about problem mentioned above (Anderson et al. 1988) complete therapeutic effect can not be expected already 7 days after application of the anthelmintic. Optimum time for sampling to verify efficiency of levamisol in one test is not supposed to be sooner than 10 to 15 days after treatment (Grimshaw et al. 1996). The author's opinion is that 7 days may be too short a period

[1] *Fascoverm Plus ®, Krka Inc., Slovenia.*

[2] *»McMaster Chamber Slide«, Merck Sharp & Dhome, NJ, USA.*

[3] *Sigma Stat ver. 3.0, Jandel Scientific, San Rafael CA, USA, 2000*

**Table 1. Reduction of faecal egg count (FEC) of particularly helmints in naturally infected sheep before (Day-7 and Day 0) and after oral aplication (Day 7 and Day 21) of closantel (10 mg kg$^{-1}$) and mebendazole (15 mg kg$^{-1}$). The test was conducted at the end of March when the flock was still in the stable. It has been proved that commonly double sampling after treatment with the use of the described mathematical-statistical model is superfluous.**

| Genus species | Day -7 n = 7 | Day 0 n = 15 | Day 7 n = 15 | Day 21 n = 15 | FEC Reduction test* (%) | | Confidence interval (95%) for Day 21 FEC Mean | | Predicted (95%) FECR% for Confidence interval on Day 21 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Arithmetical Mean† FEC/g | | | | Day 7 | Day 21 | Lower | Uper | Lower | Uper |
| *Ostertagia circumcincta* | 591 | | 0 | 6 | 99 | 99 | -1.664 | 12.754 | 98 | 100 |
| *Trichostrongylus axei* | 657 | | 0 | 2 | 100 | 100 | -1.713 | 5.284 | 99 | 100 |
| *Nematodirus filicollis* | 471 | | 79 | 0 | 83 | 100 | -0.074 | 0.228 | 99 | 100 |
| *Strongyloides papillosus Strongyloides spp* | 412 | | 10 | 5 | 98 | 99 | -1.229 | 11.229 | 97 | 100 |
| *Toxocara vitulorum* | 53 | | 0 | 0 | 100 | 100 | -0.386 | 2.386 | 95 | 100 |
| *Moniezia expansa* | 436 | | 6 | 0 | 98 | 100 | -0.106 | 0.329 | 100 | 100 |
| *Chabertia ovina* | 593 | | 2 | 0 | 100 | 100 | ‡ | ‡ | †† | †† |
| *Trichuris ovis* | 125 | | 8 | 0 | 94 | 100 | ‡ | ‡ | †† | †† |

*FECRT%= 100× [1-(FECb/FECp)] on repeated sample
† Pooled data for FECb on Day -7 and Day 0
‡ not calculated because std.dev.= 0
†† not calculated

for the anthelmintic to develop its effect to the full extent. There are still much controversial data about this question (McKenna 1997). The data in Table 1 indicate only the genus *Trihuris* is at the very bottom limit of efficiency measured by the FECR test. Grimshaw et al. (1996) pointed out in the previously mentioned study that unexpectedly 10 to 14 days after treatment the nematode eggs appear, due to the still immature adult forms of parasites in the digestive tract, on which could not be influenced during treatment. This belated wave of eggs could not be observed in this study because the second sampling was carried out three weeks after the treatment (Day 21). Consequently, the results of the FECR test, obtained in that time, are very relevant to the evaluation of efficiency of the medical treatment.

In general, there are at least four different methods available for evaluation of the extent of reduction of the FEC. Beside others, these methods differ in the use of particularly mean values (arithmetic mean versus geometric mean of average count of eggs excreted), the estimate of the faecal egg count only after treatment, the evaluation of the therapeutic effect in comparison with the control group of animals and in the degree of reduction of the FEC at which it is considered that there resistance to anthelmintics exist. The survey of many research confirm that each of the methods mentioned before has its advantages and disadvantages, at the same time (Maingi et al. 1996).

In our research the arithmetical mean was used as a measure of the average FEC value of the samples examined on the Day 7 and on the Day 21 after treatment. This is supposed to give reliable view into the dynamics of changing of the FEC. It has been proved that commonly double sampling after treatment with the use of the described mathematical-statistical model is superfluous.

## CONCLUSIONS

The therapeutic combination of closantel and mebendanzole has proved to be efficient, although the phenomenon of helmint resistance cannot be confirmed on the basis of these results. Besides, this would not be unexpected considering the large extent of resistance in other European countries. As clear technical recommendations are lacking and the strategy of the use of anthelmintics is not determined and as the Slovene drug market is small, there are favourable circumstances for the development of resistant sheep worms.

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