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Effect of combined therapy of an anthelmintic and an immunomodulator on the elimination of gastrointestinal nematodes in sheep

M. VÁRADY, A. KÖNIGOVÁ, D. ČERŇANSKÁ, J. ČORBA

Parasitological Institute of the Slovak Academy of Sciences, Hlinkova 3, 040 01 Košice, Slovak Republic,
E-mail: *varady@saske.sk*

Summary

The efficacy of the anthelmintic albendazole and the immunomodulator glucan on the elimination of gastrointestinal nematodes was investigated in naturally and experimentally infected sheep. The first experiment evaluated the effect of glucan against naturally acquired endoparasite infection. Sixty lambs were selected and divided into three experimental groups and an untreated control group, each with 20 or 10 animals. The anthelmintic used was albendazole. In all groups faecal egg counts (EPG) were performed at 1-month intervals. Results were assessed by comparing egg counts of the treated groups (albendazole, glucan, albendazole plus glucan) and the untreated control group. In the second experiment, the effect of glucan was investigated in experimentally infected groups of lambs (n=5) and worm counts of the treated and untreated group were compared. The administration of the immunomodulator showed no direct therapeutic effect, i.e. it did not reduce the level of gastrointestinal infection in both experiments. However, the combination with albendazole, in the first experiment, improved the efficacy and significant reduction in egg outputs were recorded from August to October in lambs on pastures compared to the group treated only with albendazole.

Key words: gastrointestinal nematodes; albendazole; sheep; glucan

Introduction

Wherever small ruminants are produced in the world, gastrointestinal nematodes are always present and their importance in sheep and goats is well recognized, especially because of their economic significance. The economic losses due to helminthosis include weight loss, reduced milk yield, poor feed conversion, and poor reproductive performance (see review by Hawkins, 1993).

Eradication of the majority of helminth infections is, if not possible, at least not practical. Rather the aim of parasite control is to ensure that parasite populations do not exceed levels compatible with economic production. Anthelmintic drugs have historically proven to be an effective solution for farmers with parasite problems in their animals. Unfortunately, worms developed resistance against anthelmintics, which today presents a well reported, highly prevalent problem (Waller, 2003). Resistance to all types of anthelmintic drugs has been reported (see reviews by Sangster, 1999; Jackson & Coop, 2000) and occurs in parasites of horses, small ruminants, cattle and pigs. The exacerbation of this problem over the last decade has provided the need for research into non-chemotherapeutic parasite control alternatives (Waller, 1997), or enhancing of drug efficacy by modifying the formulation or delivery mechanism of the drug (Hennessy, 1997).

Immunomodulator glucans (polysaccharide substances from bacteria, yeast or fungi) exhibit various immunopharmacological effects (e.g. stimulation of reticuloendothelial system of immunocomponent cells, antibody activity etc.). Immunorestorative effects of glucans have also been documented (Ferenčík & Bergendi, 1990). Combination of antiparasitic drugs and non-specific immunomodulators has been studied primarily from the aspect of increasing of their efficacy. The higher efficacy of combined drugs can be achieved by eliminating immuno-suppression in the host caused by parasitosis (Borošková *et al.*, 1999), drug or other exogenic factors. Glucans have also been used in the treatment of schistosomosis (Warren, 1980) and other helminthoses (Juriš *et al.*, 1992; Benková *et al.*, 1992; Borošková *et al.*, 1991; Šoltýs *et al.* 1994). Enhancement of the larvicidal efficacy of benzimidazole anthelmintics on *T. canis* larvae has been recorded in several studies (Šoltýs *et al.*, 1996; Velebný *et al.*, 1997; Hrčková & Velebný, 2001). No less important is increasing of host immunity, by making the host more resistant to re-infection. Borošková *et al.* (1995) suggest that immunomodulators may also potentiate a local immune reaction, which in turn results in a protective function against re-infection of animals on

pasture.
Therefore the present study was conducted with the aim to compare the effect of combined therapy using the albendazole and simultaneously administered glucan with the effect of albendazole or glucan alone in sheep with experimental and natural helminth infections.

Materials and Methods

Infection and experimental design
Experiment 1: Sixty lambs of Tsigaja breed, aged 3 – 8 month were used. The lambs were grazed together on unimproved pasture and belonged to a flock of about 600 sheep, which were tended by day and yarded by night. The trial lasted for a total of 20 weeks, commencing in May and ending in October. The lambs were ranked based on pre-treatment bodyweight and randomly allocated to 4 groups as follows: 10 animals in group G, 10 in group C, 20 in group A and 20 animals in group GA. A glucan formulation was administered intramuscularly at a dose of 5 mg kg^{-1} on Day 0 and Day 3 to lambs of the groups G and GA. Groups A and GA were then treated with albendazole at the recommended dose of 5 mg kg^{-1} in week 2 and week 10. Sheep in group C remained untreated to serve as controls. Individual faecal samples were collected from the rectum of each lamb at 1-month intervals to perform nematode egg counts using a modified McMaster technique, where one egg represented 50 eggs per gram of faeces (Anonymous, 1986). Larval cultures of each group of lambs were made from pooled faeces samples according to the method of Roberts and O'Sullivan (1950), using about 10 g faeces per animal, and incubated at room temperature (20 – 22°C) for 12 – 14 days. The third stage larvae were harvested and differentiated according to standard procedures (Anonymous, 1986). Body weights were measured using calibrated scales.

Experiment 2: In the second study ten worm-free Tsigaja lambs approximately 3 months age were used. On day 0 the lambs were divided at random into 2 groups of five animals (group G and group C). Lambs from group G were treated with glucan intramuscularly at a dose of 5 mg kg^{-1} at day 0, 3 and 5 (in total 15 $mg.kg^{-1}$ per animal). All 10 lambs were each inoculated with 4500 infective larvae of *Haemonchus contortus* (susceptible strain, Veterinary Laboratories Agency, UK) divided in three doses of 1500 L3 and administered at day 7, 10 and 12. From day 25 individual faecal samples were examined twice weekly for quantitative estimates of the number of *H. contortus* eggs using a modified McMaster technique (Anonymous, 1986). On day 49 all animals were humanely killed and post-mortem helminthological examination was carried out. Abomasa were immediately removed and the contents collected in individual containers. Abomasal mucosal surfaces were vigorously washed with hot tap water and the collected contents brought to a final volume of 10 l. Of this volume 1 l was poured through a 250 µm sieve and all remaining worms were collected for enumeration.

Treatments
β-glucan (carboxymethylated β-1,3-D-glucan; Mevak, Slovak Republic) was dissolved in water for injections to give a final concentration of 2 %. The lambs in experiment 1 have been treated with albendazole (Aldifal 2.5 % susp. a.u.v., Mevak, Slovak Republic) twice during the grazing season, according to the practice used on the farm.

Statistical evaluation of data
The faecal egg count reduction was determined by the method described by Coles *et al.* (1992) using the formula FECR % = 100 × (1 – T/C), where T and C are the arithmetic means of the number of eggs in the treated (group G, GA) and control group. Wilk-Shapiro test were used to describe the distribution of faecal egg counts and worm count data. The *t*-test was used for statistical evaluation of differences between groups having normally distributed data and the Mann-Whitney test was used on data with non normal distribution (GraphPad Prism 3.02, GraphPad Software, SanDiego, USA).

Results

Experiment 1: The changes in faecal egg count (eggs of strongyles and *Nematodirus* spp. eggs) over the duration of the trial are summarised in Fig. 1. At the beginning of experiment the mean faecal egg counts were not significantly

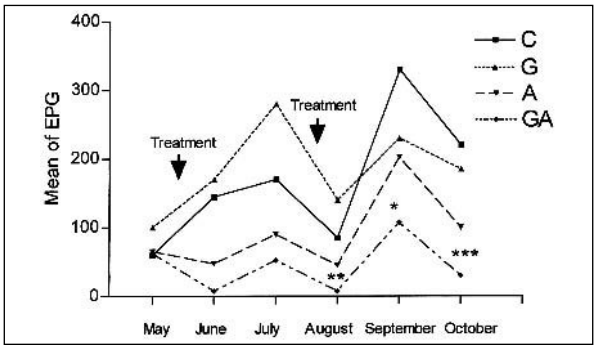

Fig. 1. The mean faecal egg counts (EPG) during pasture period in lambs of four experimental groups. Statistical significance between groups A and GA: $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$

Table 1. The mean reduction of egg counts in experimental groups during grazing season (Experiment 1)

Group/Months	June	July	August	September	October	Mean reduction (%)
G	-6	-64	-64	24	16	-18.8
A	70.4	70.4	47.1	48	32.9	53.8
GA	95.4	69.2	91.2	77.1	86.4	83.9

different between groups ($P > 0.05$). After treatment with albendazole, egg counts in groups A and GA were reduced compared to untreated controls (group C) and the group that received only glucan formulation (group G). The lowest egg output during the whole study period was observed in group GA. Statistical differences ($P < 0.01$; $P < 0.05$; $P < 0.001$) between egg outputs in groups GA and G were recorded from August to October. The mean reducetion in faecal egg counts in group GA was 83.9 %, compared to 53.8 % in group A (Table 1).

Cultures of the bulked faeces from the experimental groups of lambs indicated that the lambs were infected with helminths of the following genera of the order Strongylida: *Teladorsagia* spp., *Oesophagostomum* spp., *Chabertia ovina*, *Strongyloides papillosus*, *Bunostomum trigonocephalum*, *Trichostrongylus* spp., *Cooperia* spp., *Nematodirus* spp. and *Haemonchus contortus*. The species encountered through larval cultures were mainly *Teladorsagia* spp. and *Trichostrongylus* spp., with the first species predominating at the beginning of grazing season and the latter at the end. Table 2 shows mean body weight and weight gain until October. Weight gain was highest in group GA, however the difference compared to control group was not significant ($P > 0.05$).

Table 2. Mean body weight and weight gains in experimental groups (Experiment 1)

Group	Weight 1*	Weight 2**	Weight gain
C	14.65 ± 1.85	26.9 ± 4.0	12.25
G	16.4 ± 4.14	28.15 ± 6.43	11.75
A	14.32 ± 1.73	25.39 ± 2.62	11.07
GA	14.73 ± 2.68	27.86 ± 3.99	13.13

*mean body weight of the lambs at the beginning of the experiment;
**mean body weight of the lambs at the end of the experiment

Experiment 2: The mean faecal egg excretion in groups C and G is illustrated in Fig. 2. Mean worm counts for the two infected groups are presented in Table 3. No significant difference was observed for numbers of male or female worms or total *H. contortus* numbers.

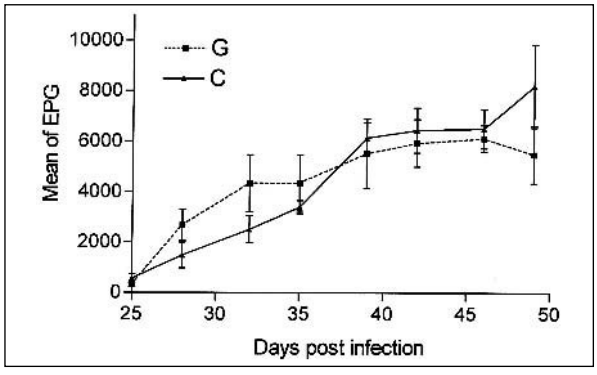

Fig. 2. Patterns of egg excretion (EPG) in Experiment 2 of lambs infected with *Haemonchus contortus*. Egg counts are in arithmetic means ± SD

Table 3. Worm burdens ± SD of *Haemonchus contortus* of the lambs in Experiment 2

Group G	Group C
980	1345
2080	890
1600	2870
2310	620
1120	820
1618 ± 580	1742 ± 1312

Discussion

The principal findings from these trials were that lambs receiving combined therapy with an anthelmintic albendazole and an immunomodulator (glucan) showed a significant reduction of faecal egg output in natural infections.

The results suggest that, to restore the host's immune status after the administration of benzimidazole drugs, glucan may be used in order to enhance the effect of the anthelmintic. Glucans are known to stimulate natural and specific immunity by activating cells of the mononuclear phagocytic system. There are several studies, which have documented the positive effect of glucan on the immune system of hosts suffering from parasitic infection. However, the majority of these experimental works investigated the effect of the immunomodulator, either with combination with drugs or alone, on tissue parasites such as *Toxocara canis* or *Mesocestoides corti*. Our study demonstrates the effect of glucan on re-infection with lumen dwelling parasites of the gastrointestinal tract. The mucosal immunity of hosts infected with gastrointestinal worms can differ slightly from the immune-response of animals infected with tissue migrating larvae. However, only a limited number of data is available on the restorative effect of glucan in small ruminants infected by gastrointestinal nematodes. The study of Borošková *et al.* (1995) confirmed the protective function of glucan against reinfection of nematodes in lambs on pasture, resulting in a lower prevalence and intensity of gastrointestinal nematode infection compared to untreated animals. However, their experiment differs in two principal respects from the one reported here. Firstly, the glucan was combined with IgG and zinc and secondly immunomodulator was given to lambs in four repeated doses.

In our study we have used two (Experiment 1) or three (Experiment 2) doses of glucan. It has been shown by several authors (Hamuro *et al.*, 1980; Ditteová *et al.*, 2003) that during the long-term course of infection, the most effective schedule is administration of 5 doses of glucan (25 mg kg^{-1} BW).

The application of glucan has no effect on either egg counts or worm burdens of *H. contortus*, as was confirmed in both experiments. In contrast, the application of glucan with albendazole significantly reduced the egg output of grazing animals compared to the group receiving only anthelmintic treatment. This suggests that glucan could eliminate immunosupression in the host caused by albendazole,

because the benzimidazole drug may induce adverse effects in the immunostatus of the infected host (Ozeretskovskaya, 1980).

The composition of larval cultures from all groups showed a majority of *Teladorsagia* spp. in the beginning of the study. This could be the result of anthelmintic selection, because the egg count reduction in group A after treatment only slightly reduced.

In conclusion, in our study the most efficient treatment schedule regarding the reduction of egg counts of lambs on the pasture seems to be administration of glucan with anthelmintic. However, more detailed immunological studies are needed to prove the immunomodulative effect of glucan in parasitized small ruminants on pasture.

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