Revista Ibérica de Parasitología, 1982. Vol. Extra, pp. 543-553

CRITICAL EXPERIMENTS WITH ALBENDAZOLE IN THE TREATMENT OF PROTOSTRONGYLID INFECTION OF SHEEP

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SUMMARY

In a previous paper ABZ was shown to have efficacy against Protostrongylids of sheep. In the present paper the results of a critical test involving 60 sheep are given. The experimental sheep were divided into 3 groups G-1 controls; G-2 treated with a single dose of 10 mg/kg I.w.; G-3 treated with two 7,5 mg/kg I.w. doses.

Efficacy was calculated *in vivo* by means of 5 pre-treatment and 6 post-treatment faecal analyses and *Post-mortem* by counting L-I in the rigth lung and by macro- and microscopical studies of lesioned areas (left lung).

In the 1st and 2nd post-treatment weeks, the decrease of L-I in faeces reached 32.35-89 3 % in G-2 sheep, and 62.86-90.8 % in the G-3 ones. However, the linear correlation indexes between data of larval elimination of different groups were not very conclusive for ABZ, while previous experiments gave statistically significative results.

From the L-I count and necropsy, the efficacy was shown to be 44.7 % in the G-2 sheep, and 81.3 % in the G-3 ones.

The quadricular-gage study of the affected areas showed that parasitized areas represented about 16.7-18.0 % of the whole surface. A highly significant correlation was observed to exist between those areas and the pre-treatment elimination of L-I, but not after treatment. At the same time, the finding of L-I on affected areas was higher on the control than

in the treated animals. The conclusion is that the reparation of the lesions requires more time than the period studied in the treated sheep as well.

The difficulties of evaluation of anthelmintics against Protostrongylid are also discussed.

RESUMEN

El trabajo da a conocer los resultados de una experiencia crítica con 60 ovejas, divididas en 3 lotes: G-1, testigos; G-2, tratadas con una sola dosis de 10 mg/kg de peso vivo, de ABZ; G-3, tratadas con dos dosis de 7.5 mg/kg. La eficacia se calculó en vivo por medio de 5 análisis fecales previos al tratamiento y otros 6 posteriores al mismo, más el recuento de L-I *post mortem* en el pulmón derecho y estudios macro y microscópicos de las áreas lesionadas del pulmón izquierdo.

En las primera y segunda semanas post-tratamientos, el descenso de L-I en las heces fue del 32,35-89,3 % en el G-2, y del 62,86-90,8 % en el G-3. No obstante, los índices de correlación linear entre los datos de la eliminación larvaria de los diferentes grupos no fueron muy concluyentes en favor del ABZ, aunque en experiencias previas sí se obtuvieron resultados significativos.

Considerando el recuento de L-I en pulmón y los datos de necropsia, la eficacia resultó del 44,7 % para el G-2 y del 81,3 % para el G-3.

El estudio de las áreas afectadas en el pulmón, mediante una plantilla cuadriculada para valorar la extensión de las lesiones, mostró que las áreas parasitadas representaban en torno al 16,7-18,0 % de la superficie total. Se apreció correlación altamente significativa entre la amplitud de estas áreas y la eliminación larva previa al tratamiento, pero no en los días posteriores a la medicación. Se hallaron más larvas en las zonas enfermas, en los animales testigos que en los tratados, pero se llega a la conclusión de que la reparación de las lesiones requiere más tiempo que el período estudiado en esta experiencia.

Se analizan las dificultades de evaluación de antihelmínticos contra Protostrongylidos.

INTRODUCTION

The treatment of verminous broncho-pneumonia caused by Protostrongilids is extremely difficult. The action of some benzimidazols seems to be important (Düwel, 1978; Kutzer et al., 1974; Gutierrez et al., 1979; Ramisz et al., 1979; Cordero del Campillo et al., 1980). However, given the wide variety of criteria used in evaluation, it is very difficult to compare results ob-

tained by different authors. This work investigates the action of Albendazole (ABZ) (VALBAZEN, a registered trade mark of Smith-Kline) and discussed some aspects concerning the assessment of its effectiveness, as well as offering a method to determine the extent of lung damage.

MATERIALS AND METHODS

Animals. Sixty specimens were selected from a flock of 250 free-grazing "churra" sheep. All had been grazing for more than two years, and were chosen for their similar levels of parasites. They were randomly divided into three groups of 20, G-I, G-II, and G-III.

Coprology. Five faecal analyses were carried out (following the BAERMANN-WETZEL method), 20, 14, 9 and 3 days before treatment as well as the day when treatment was administered. All the Protostrongylids L-I were identified together without distinguishing between the genera, although *Muellerius* and *Cystocaulus* spp. were found to predominate, in that order.

Treatment.—ABZ was used in a 1.9 % solution. The control group (G-I) was given two dosis of bovine milk as a placebo, with a week's interval between the dosis. The G-II group was given one dose of the activ product in a proportion of 7.5 mg/kg live weight with a week's interval between the dosis. The preparation was given orally by means of a dosifying gun.

Control of results. Coprological analyses were carried out on all the animals 1, 2. 8, 9, 14 and 21 days after the first treatment.

23 days after the first treatment 10 animals from each group were killed, while the rest were killed 29 days after treatment. In the necropsy, nodules containing parasites were removed from the right lung. These nodules were halved longitudinally, one half being kept for histological examination while the other was cup up with scissors before putting it in BAERMANN and lukewarm water (15 - 20° C). In this way, L-I were obtained by migration. The number of larvae obtained was multiplied by two to find the total number in the lung.

The left lung was used to carry out scaled drawings of the lung outline. Both faces were drawn by means of a template

made up of 308 equal squares for the ventral face and of 314 for the dorsal face (622 in all; Fig. 1). Six incisions (5 dorsal and 1 ventral) were made in each of the larval nodules, followed by a scrape, then these were examined under the microscope in covered slides to discover the presence of eggs and/or larvae. The results were analysed statistically.

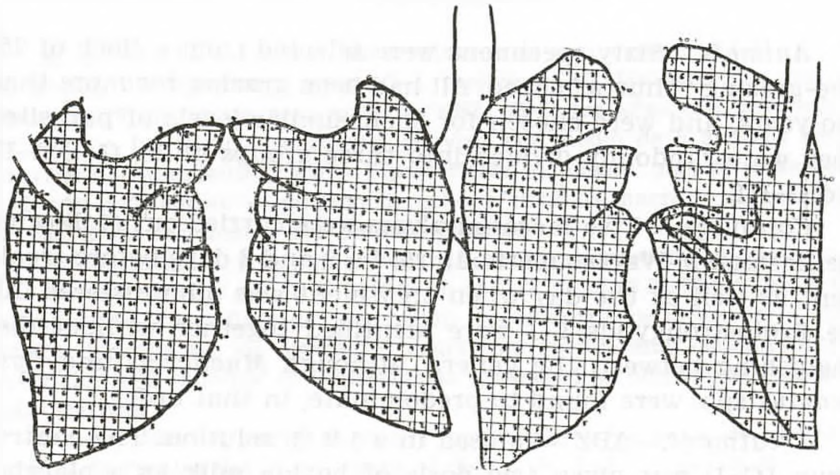

Fig. 1

RESULTS AND DISCUSSION

Coprology. Results of the faecal analyses (Table I) show that the reduction in the number of larvae varied, depending on the lengths of time considered, the average for the first and second days after treatment was 46.51 % for G-II and 69.77 % for G-III. In the following period the average was 91.52 % for G-II and 92.41 % for G-III. Taking into account all the averages the reduction in the number of larvae was 77.05 % for G-II and 85.00 % for G-III.

Given that we detected a spontaneous reduction in the number of larvae in the control group it would seem advisable to evaluate the effectiveness of ABZ using data for larval expulsion calculated by a direct proportionality rule. Following this method (Table II), the G-II reduction was 32.35 % and

LARVAE (L/g) IN FAECES

Days	Group I (Control) L/g	$\bar{x}$	Albendazole (Group II) 1×10 mg/kg L/g	$\bar{x}$	Reduct. %	Albendazole (Group III) 2×7,5 mg/kg L/g	$\bar{x}$	Reduct. %
Pretreat.								
20	40		62			18		
14	34		44			42		
9	66	92	36	73		122	75	
3	229		149			139		
0	88		74			55		
Post-treat.								
1	56	43	30	23	46.51	23	13	69.77
2	29		16			3		
8	42		1			1		
9	24	44.8	1	3.8	91.52	0.6	3.4	92.41
14	44		3			0.3		
21	69		10			12		
Post-treat. mean	44		10.1		77.05	6.6		85.00

TABLE I

MEANS OF ACTUAL AND CALCULATED NUMBER OF L/g

Days	(Group I)	Albendazole (Group II)	(Group III)
Pretreament (5 analyses)	92	73	75
Post-treatment (2 analyses after 2nd treatment)	43	23 /34 (*)	13 /35 (*)
Post-treatment (4 analyses final)	44.8	3.8/35.5 (*)	3.4/36.5 (*)
Post-treatment mean	44.0	10.1/34 9 (*)	6.6/35.8 (*)

(*) First value: mean number of larvae actually found.
Second value: calculated number of larvae.

TABLE II

that of G-III 62.86 %, in the two days following the initial treatment. After two weeks the G-II reduction was 89.30 % and that of G-III 90.70 % (at this stage G-III had received a second treatment). The global/overall averages were 71.0 % for G-II and 81.0 % for G-III.

In order to find out whether these were freag results, or due to the treatment, it was decided to determine the levels of lineal correlation between the data concerning larval expulsion in the three groups. The results were as follows:

$$\text{G-I / G- II: } r = 0.9545 \quad (P < 0.05)$$
$$\text{G-I / G-III: } r = 0.9869 \quad (P < 0.02)$$
$$\text{G-II / G-III: } r = 0.9715 \quad (P < 0.05)$$

It can be seen that there is a close correlation between the different groups, and there is little margin to demonstrate the possible effectiveness of ABZ, in the conditions of this experiment. The correlation showing a decrease in the number of larvae is highly significative between all the groups. Fig. I shows larval expulsion in the groups.

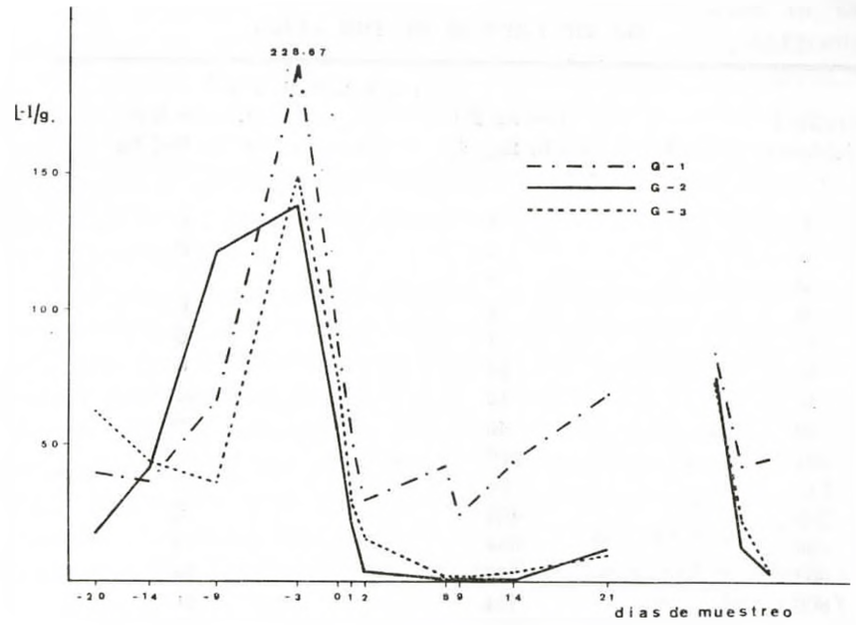

Fig. 2

Right lung: larvae found. The reduction in the number of larvae found was 56.09 % in G-II and 84.78 % in G-III (Table III).

If we follow the method used with the coprological results in order to correct both possible difference caused by the different parasite burdens in the groups and spontaneous tendencies resulting in variations, if we had given the sheep no course of treatment, we would expect to find an average number of 3,034 larvae in G-II and 3,117 in G-III (given an average number of 3,824 in G-I). Therefore, the efficacy would be 44.7 % in G-II and 81.3 % in G-III.

Left lung: areas of larval nodules. The 622 squares of the left lung template gave us a total of 12,440 squares for each group of twenty animals. In G-1 we found 2,245 squares with parasites (18 % of the total number), 2,083 (16.7 %) for G-II and 2,112 (16.98 %) for G-III. These values are closely linked to the results for larval elimination before treatment ($r = 0.9974$; $P < 0.01$).

Nr. OF LARVAE IN THE LUNG					
		Albendazole			
Group I (Control)	$\bar{x}$	(Group II) 1×10 mg/kg	$\bar{x}$	(Group III) 2 × 7.5 mg/kg	$\bar{x}$
0		0		0	
0		0		0	
0		0		0	
6		0		6	
48		0		6	
84		12		6	
84		18		12	
120		48		12	
120		216		12	
132		324		12	
252		468		12	
468		504		18	
2.808		720		24	
3.600		734		24	
5.760		1.728		48	
8.640		2.232		120	
9.000		3.168		168	
12.960		3.600		1.224	
14.400		9.720		1.656	
18.000	3.824	10.080	1.678	8.280	582
Reduction			56.09 %		84.78 %

TABLE III

Examination of the larval nodules, by means of a scrape carried out on a previously cut would, showed that 46.67 % were positive for G-I, 35.00 % for G-II and 17.5 % for G-III. That means that efficacy calculated on these data would be 25.0 % for G-II and 62.5 % for G-III.

It is evident that varying conclusions as to the efficacy of ABZ may be drawn depending on the results of the different parameters considered (e.g., coprology, larvae obtained from the lung, microscopic examination of scrapes from larval nodules, extension of the affected areas etc.).

The problems of systematizing techniques used in the assessment of helminths were analysed at the 9th Conference of the World Association for the Advancement of Veterinary Parasitology (Budapest, July 13-17th, 1981). A committee of experts proposed agreements it had reached concerning various host/parasite systems, but it remained clear that a methodical approach to the assessment of anthelmintics on Protostrongylids has yet to be found.

The small lung worms present us with great difficulties in the assessment of effective anthelmintic products. Coprological data are not reliable because firstly, larval elimination varies greatly between individuals due to the different levels of reproductive activity among the adults, and the ways in which the host reacts (with the possibility of temporary occlusion of the small bronchioli and alveoli). Secondly, there are pronounced seasonal variations (Cabaret et al., 1981; Morrondo et al., 1978; Ramirez, 1967; Reguera et al., 1979). It is necessary to carry out the tests at the time of year which guarantees the greatest stability in larval elimination (this requires a knowledge of the epizootiology of Protostrongylid infection in the area). Thirdly, if GEVONDIAN's observation (1969) are correct, the time of year has an impact on L-I resistance. Finally, we must take into account possible variations caused by host's age. As yet it is not known wheter this factor influences larval elimination, although Morrondo et al. (*ibid.*) have found these factors to be related. What is obvious is that all experiments must be studied over a long period of time.

Coprological results have been shown to be of doubtful value, but a live larvae count carried out on the lung is more trustworthy. As with other nematodes, it may be that ABZ is more effective against adult worms but there are great problems in finding and counting the adults. Techniques used at present (including artificial digestion) have not given us satisfactory results (unpublished ata).

Only slight differences were found in the healing of lung lesions during the time studied (23 - 29 days after treatment). Longer periods are undoubtedly needed to obtain a greater degree of healing. Nevertheless, the system may be used in longer experiments to assess metrically the evolution of the

lesions. FORRESTER (1971) calculated the damaged surfaces in mm^2 in his experiments.

ABZ has confirmed its qualities in combating Protostrongylids (CORDERO et al., *ibid*; QUIROZ and RODRIGUEZ, 1980; HIMONAS, pers commu., 1980), but at present it would seem necessary to conduct further experiments using larger dosages and more treatments. It is also vital to formulate a standard method to assess the action of anthelmintics on Protostrongylids.

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