The efficacy of mixtures of albendazole sulphoxide and levamisole against sheep nematodes resistant to benzimidazole and levamisole

N ANDERSON, *PJ MARTIN * and RG JARRETT †

SUMMARY: Faecal egg count reduction tests and an anthelmintic efficiency assay were used to assess the efficacy of combinations of albendazole sulphoxide and levamisole against populations of Ostertagia and Trichostrongylus sp. which contained different proportions of worms resistant to both benzimidazole and levamisole anthelmintics. Compared to the effects of either drug alone, significantly greater efficacy was obtained using combinations which included dose rates similar to those recommended for the separate components. At these dose rates, the mixtures reduced mean faecal egg counts by 95% or more, and caused a reduction of 68% in adult *Ostertagia* sp. and more than 95% for 4th stage *Ostertagia* and *T colubriformis*. The increased efficacy of the mixtures could be accounted for by actions of the drugs acting independently.
Aust Vet J 68: 127–132

Introduction

Simulation studies for the control of insect populations by pesticides show that strategies using mixtures of compounds with different modes of action can delay the development of resistance for periods several-fold greater than those using the same compounds either sequentially, or in rotation (Curtis 1985; Mani 1985). Assuming the absence of cross-resistance to the compounds, and a dose rate which effectively removes heterozygotes, the delay is due to the low occurrence of individuals homozygous for the genes for resistance to both compounds. Logically, a strategy using mixtures is best implemented when the frequency of resistance genes is low because of the rare likelihood of homozygous individuals.

For similar reasons, mixtures of anthelmintics may have a role in delaying the development of resistance to the broad-spectrum compounds (Bennet *et al* 1980; Anderson *et al* 1988), although their value when resistance is widespread is not known.

Drugs given together may have an additive action, or show antagonism or synergism, the response is either less or greater than the additive effects of one component. Evidence for synergism between mebendazole and levamisole was presented by Bennet *et al* (1980). At dose rates of one seventh that of the minimum effective dose for levamisole, and 40-fold greater than that for mebendazole, neither drug significantly reduced a population of mebendazole-resistant *Haemonchus contortus*, but together caused a 60% reduction in total worm count.

The objectives of the present study were to assess the efficacy of combinations of albendazole sulphoxide and levamisole against strains of nematodes, representative of current field populations, to determine an effective dose rate for field use and to determine whether the effects were synergistic or additive.

Materials and Methods

Three experiments were undertaken on sheep experimentally infected with prepared strains of *Ostertagia* spp and *Trichostrongylus colubriformis*. In the first experiment, 3 dose rates, namely, zero, one third and two thirds of the recommended dose rates of albendazole sulphoxide and levamisole, either singly or in combination, were assessed according to the 3 x 3 factorial design shown in Table 1.

From a flock of 120 lambs experimentally infected with *Ostertagia* spp and *T. colubriformis*, 9 groups of 12 sheep, were taken. The treatments set out in Table 1 were given 26 d after infection of the sheep and faecal worm egg counts on samples taken at this time and 10 d after treatment, provided the data for assessing treatments. Species differentiation of larvae from cultures prepared for each group pre- and post- treatment were used to determine the effects of treatment on each species of nematode.

In the second experiment, several combinations of the 2 drugs were tested to determine an effective dose for field use. A flock of 112 lambs was infected with *Ostertagia* spp and *T colubriformis* and allocated to 9 treatment groups, each of 12 sheep. Sheep in group 1 remained untreated, whereas those in the other 8 groups were given the treatments set out in Table 4 on day 25 after infection. Pre- and post-treatment egg counts were used to assess the efficacy of the treatments.

Experiment 3 was a controlled anthelmintic efficiency assay (Moskey and Harwood 1941; Clark and Turton 1973), using 2 dose combinations of albendazole sulphoxide and levamisole and each drug separately. Forty lambs were infected with *Ostertagia* and *Trichostrongylus* larvae and 25 d later were allocated to 5 equivalent groups. Sheep in group 1 remained untreated, those in group 2 were given 4 mg/kg albendazole sulphoxide, those in group 3, 7.5 mg/kg levamisole, those in group 4 a mixture of 3.6 mg/kg albendazole sulphoxide and 7.5 mg/kg levamisole, and sheep in group 5 were given a double dose of the combination, namely, 7.2 mg/kg albendazole sulphoxide and 15 mg/kg levamisole. Faecal egg counts were obtained pre- and post-treatment and the sheep were killed for worm counts 12 d after treatment.

TABLE 1
Treatment groups for various dose combinations of albendazole sulphoxide and levamisole used in experiment 1

Anthelmintic		Albendazole sulphoxide (mg/kg)		
		0	1.3	2.7
Levamisole (mg/kg)	0	G1	G2	G5
	2.3	G3	G4	G8
	4.5	G6	G7	G9

* CSIRO Division of Animal Health, Animal Health Research Laboratory, Private Bag No.1, PO, Parkville, Victoria 3052
† Statistical Consulting Centre, University of Melbourne, Parkville, Victoria 3052

Animals

Newly weaned, 4 to 5-month-old Merino or Corriedale lambs were treated with ivermectin* on arrival at the field station, housed in sheds and fed hay ad libitum throughout the experiments. Animals were assigned to equivalent groups using body weight and faecal egg counts obtained 4 d before treatment as determinants for allocation.

Nematode Strains and Infections

The strains of *Ostertagia* spp and *Tcolubriformis* used for these studies were derived from field isolates with known responses to benzimidazole and levamisole anthelmintics.

Ostertagia spp BRO87 were isolated in 1987 from sheep on farm A in the Western District of Victoria (Anderson *et al* 1988) and showed resistance to benzimidazole and levamisole. The BCRT80 strain of *T colubriformis* originated from the McMaster Field Station at Badgery's Creek, New South Wales, and had been selected with levamisole in the laboratory (Waller *et al* 1985). This strain has a high degree of resistance to levamisole but is susceptible to benzimidazoles. To obtain a strain with multiple resistance, larvae of BCRT80 and the benzimidazole resistant KRT81 were mixed 60:40, respectively, and used to infect a worm-free sheep. Larvae from this infection and from the BRO87 strain of *Ostertagia* spp were used to infect sheep in experiment 1.

To increase the proportion of nematodes resistant to both compounds, additional sheep were infected with either *Ostertagia* or *Trichostrongylus* spp larvae from the same batch used in experiment 1 and 25 d later were treated with a mixture of one-third the recommended dose rates of albendazole sulphoxide and levamisole, 1.33 and 2.26 mg/kg, respectively. Eggs passed after treatment were cultured for larvae which were used in experiments 2 and 3. Sheep in experiment 1 were dosed with 30,000 *Ostertagia* and 15,000 *T colubriformis* larvae and those in the other experiments received doses of 12,000 and 5,000 larvae, respectively, of the 2 species.

The benzimidazole susceptible KS79 and resistant KR79 strains of *Ostertagia* spp and comparable strains of *Tcolubriformis*, KST80 and KRT81, respectively, were used as standards in egg-hatch assays for benzimidazole resistance. The origin, history and characteristics of these strains have been previously reported, Martin *et al* (1982, 1984).

Anthelmintics and Doses for Sheep

Commercial formulations of albendazole sulphoxide† and levamisole‡ were used for all treatments which were administered orally, using 10 ml disposable syringes. The doses were computed from the body weight of each sheep, and for levamisole, have been expressed in amounts of levamisole base. Appropriate dilutions with water were made for each compound to ensure approximate equivalence in the dose volume given to each animal.

Worm Egg Counts, Larval Culture and Species Differentiation

The method for worm egg counts described by Anderson (1968) was used. Briefly, 3 g of faeces were homogenised in 42 ml of water. The faecal suspension was passed through a 60 mesh sieve to remove coarse material and the liquid poured into a 15 ml tube which was centrifuged at 2,000 RPM for 3 to 4 min. The liquid was siphoned off and the plug of faecal debris resuspended in a saturated solution of sodium chloride. After thorough mixing, 2 chambers of the Universal McMaster Slide§ were filled and the eggs counted at x 40 magnification. Each egg counted was equivalent to 15 epg of the original faecal sample.

A further 3 g from each sample of faeces were pooled to form a bulk culture for each treatment group. After 8 d incubation at 21±1°C, infective larvae were recovered and up to 200 were classified as either *Ostertagia* or *Trichostrongylus* spp using the morphological criteria of Douvres (1957)

For worm counts standard procedures and methods based on those described by Anderson (1972) were used.

In-vitro Egg-hatch Assays

The degree of resistance of all strains to benzimidazole compounds was determined from egg-hatch assays using the methods described by Le Jambre (1976) and Martin *et al* (1982).

Analysis

All counts were transformed to logarithms after half the dilution factor (7.5 for worm egg counts and 10 for worm counts) was added to zero values, and an analysis of variance was used to determine differences between treatments and the interaction between the drugs.

The main effects for the two drugs indicate the effects of different doses and the interaction tests the additive model in which the two drugs act independently of one another. A significant interaction term indicates either synergism or antagonism. If the 2 drugs act independently, and at the dose rates given, reduce counts relative to the control value by proportions p_1 and p_2 for albendazole sulphoxide and r_1 and r_2 for levamisole, then groups G4, G7, G8 and G9 in Table 1 would be expected to yield proportions p1 r1, p1 r2, p2 r1 and p2 r2, respectively.

Separate analyses were conducted on log transformed post-treatment counts and the difference between the transformed pre- and post-treatment counts. The latter analysis was undertaken to minimise the between-sheep variation. The method of calculating percentage reduction and the 95% confidence intervals was based on arithmetic mean counts as described in Appendix 1. Results from the egg-hatch assays were analysed according to the method used in Martin et al (1982).

Results

Egg-hatch Assays

The 50% effective dose, ED50, and resistance ratio of the strains used to infect sheep in the 3 experiments are shown in Table 2. Both

TABLE 2

The ED_{50}, its standard error and resistance ratio calculated for in-vitro egg-hatch assays on the strains of *Ostertagia* and *Trichostrongylus* spp used in experiments 1, 2 and 3

		In-vitro Egg Hatch Assay	
		ED_{50}	Resistance
Nematode	Strain	± S.E. µg/ml	Ratio
Ostertagia spp	KS79	0.08 ± 0.004	1
	KR79	0.98 ± 0.02	12.3
	BR087 (Exp 1)	0.23 ± 0.01	2.9
	BR087 (Exp 2&3)*	1.46 ± 0.08	18.3
Trichostrongylus colubriformia	KST81	0.04 ± 0.005	1
	KRT81	0.78 ± 0.02	19.5
	BCRT80/KRT81 (Exp 1)	0.11 ± 0.008	2.8
	BCRT80/KRTS1 (Exp 2&3)	1.03 ± 0.09	

* Measured after culture sheep had been treated with a one-third recommended dose of both albendazole sulphoxide and levamisole

* Ivomec liquid for sheep containing 0.89 g/l ivermectin, Merck, Sharp and Dohme Australia Pty Ltd, Sydney, New South Wales.

† Rycoben, containing 40 g/l albendazole oxide. Youngs Animal Health, Pty.,Ltd., Blackburn, Victoria.

‡ Ringer Low Volume Levamisole Oral Anthelmintic, containing 80 g/l levamisole hydrochloride, Youngs Animal Health, Pty Ltd., Blackburn, Victoria.

§ J.A. Whitlock and Company, Sydney, New South Wales

TABLE 3

Arithmetic mean counts of total, *Ostertagia* and *Trichostrongylus* eggs 10 d after treatment, percent reductions and 95% confidence limits from 9 groups of sheep in experiment 1

Group treatment mg/kg	Total eggs Mean	% Reduct.	95% Limits	Ostertagia eggs Mean	% Reduct.	95% Limits	Trichostrongylus eggs Mean	% Reduct.	95% Limit
1. Controls	2399	NA	NA	456	NA	NA	1943	NA	NA
2. RBZ* 1.3	400	83	68,91	272	40	0,69	128	93	87,98
3. LEV† 2.3	473	80	65,89	184	60	29,77	288	85	74,92
4. RBZ + LEV 1.3 + 2.3	284	88	78,94	258	43	0,70	26	99	98,99
5. RBZ 2.7	270	89	80,94	230	50	9.72	41	98	96,99
6. LEV 4.5	277	88	81,93	72	84	74,90	205	89	83,94
7. RBZ + LEV 1.3 + 4.5	73	97	94,98	42	91	82,95	30	98	97,99
8. RBZ + LEV 2.7 + 2.3	113	95	91,98	101	78	58,88	11	99	99,100
9. RBZ + LEV 2.7 + 4.5	44	98	96,99	36	92	84,96	8	100	99,100

* RBZ = ricobendazole = albendazole sulphoxide
† LEV = levamisole

the Ostertagia and T colubriformis strain used in experiment 1 showed a moderate degree of resistance to benzimidazole drugs. However, after simultaneous treatment with one-third of the recommended doses of albendazole sulphoxide and levamisole, resistance ratios for both species increased markedly, and are comparable to those of the reference resistant strains KR79 and KRT81.

Experiment 1 - A test for synergism

The arithmetic mean counts of total *Ostertagia* and *Trichostrongylus* spp eggs 10 d after treatment, the percentage reductions, and 95% confidence limits for the 9 treatment groups are set out in Table 3. Adjustment for pre-treatment counts made little difference to the results.

Treatment with doses of one-third the recommended rate of albendazole sulphoxide and levamisole, 1.33 and 2.26 mg/kg, respectively, either alone or together, caused reductions in total egg counts of greater than 80%. The two-thirds recommended dose of each compound produced similar results, and the extensive overlap of the confidence intervals from all of these groups indicates no significant difference between them. The combination of one-third albendazole sulphoxide and two-thirds levamisole and vice versa, (groups 7 and 8), and the two-thirds dose of each compound, group 9, produced reductions of 95 to 98% which were significantly higher than those for the other groups.

From an inspection of Table 3, it can be seen that *Ostertagia* spp were resistant to both compounds at both dose rates. Furthermore, combinations which included the higher dose of levamisole, 4.52 mg/kg, were superior to that which included the lower dose of levamisole. Some degree of resistance to levamisole was shown by *Trichostrongylus* spp but this population was essentially susceptible to albendazole sulphoxide.

Figure 1 shows the arithmetic mean counts for the 9 treatment groups plotted on a log scale. If the drugs act independently, the 3 lines should be parallel corresponding to a constant percentage reduction as the dose rate of levamisole increases, regardless of the dose rate of albendazole sulphoxide. In the formal test, by analysis of variance, the main effects of the 2 drugs were highly significant (P), but the interaction (F4,99=2.13) was not.

Experiment 2 - A dose response of different combinations of the two drugs

Percentage reductions and their 95% confidence limits for total, *Ostertagia* and *Trichostrongylus* spp eggs for each of the 9

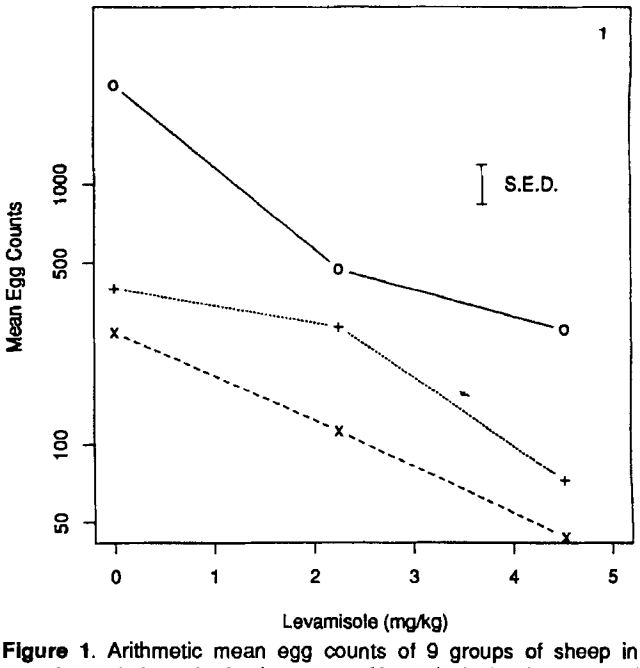

Figure 1. Arithmetic mean egg counts of 9 groups of sheep in experiment 1. At each of 3 dose rates of levamisole the dose rates of albendazole oxide were 0 (0-0), 1.3 mg/kg (+.....+) and 2.7 mg/kg (x----x).

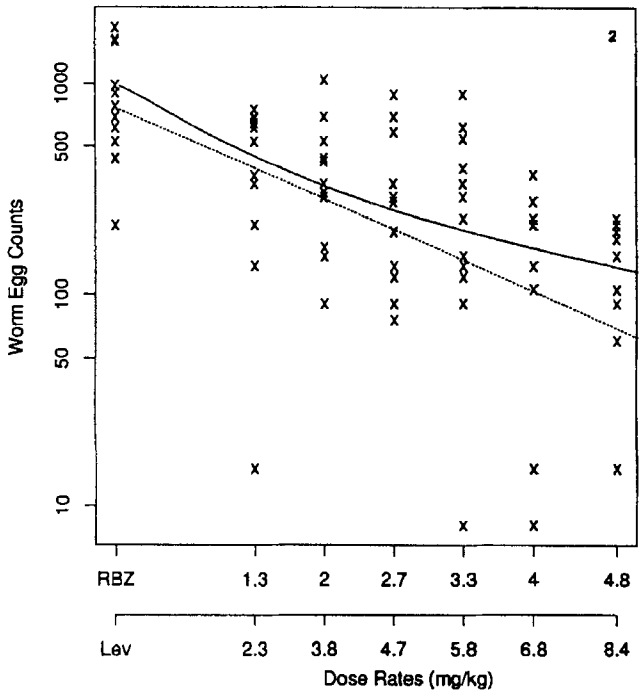

Figure 2. The relationship between log post-treatment egg counts and doses of mixtures comprising different proportions of the recommended dose rates for albendazole sulphoxide (RBZ) and levamisole (Lev). Fitted lines for the exponential decay and logistic models are also shown.

groups in experiment 2 have been set out in Table 4. The greatest reductions in total egg counts, 86 and 88%, were obtained from the 2 highest concentrations of the 2 compounds.

In Figure 2, the post-treatment counts of untreated sheep, and those given the combined treatments, have been plotted on a logarithmic scale against units of the recommended dose rates of both drugs. The relationship between counts and dose was examined, using both the logistic and exponential decay models and the fitted lines are shown, also.

The fitted line from the exponential decay model is straight because the counts have been shown on a logarithmic scale. The slope of this line, -2.01, indicates that log post-treatment counts fall by 2.01 units for every unit of dose which, in this case, corresponds to 4 mg/kg albendazole sulphoxide and 7.5 mg/kg levamisole. The number of dose units to cause a 90% reduction in post-treatment counts was calculated to be 1.15 with a standard error of 0.18, and for a reduction of 95% the values are 1.49 and 0.26, respectively. Therefore, it can be estimated that for a reduction of 90% in egg counts of the strains used in experiment 2, 4.6 mg/kg albendazole sulphoxide and 8.63 mg/kg levamisole would be needed in the mixture. Comparable values for a 95% reduction would be 5.96 and 11.18 mg/kg, respectively.

The logistic curve also gave a good fit to the data, (Figure 2), but it can be seen that the incremental decrease is much lower at the right hand end because of the large variation in counts between sheep in groups receiving the 2 highest dose rates. Consequently, the estimated dose at which this curve falls to 10% of its initial value, a 90% reduction in egg count, is 1.57 dose units or 6.3 mg/kg albendazole sulphoxide and 11.7 mg/kg levamisole.

A test for interaction was done by analysis of variance on log-transformed counts from sheep in groups 1, 6, 8 and 9 but the variance ratio ($F_{1,44}=1.84$,) was not significant at the 5% level of probability.

Experiment 3 - Anthelmintic efficiency assay

Mean post-treatment egg counts, percentage reduction and 95% confidence limits for the 5 groups of sheep in experiment 3, are given in Table 5. Reductions in egg count caused by the single and double doses of the drug mixture were significantly greater than those for each compound separately. The geometric mean

TABLE 4

Arithmetic mean counts of total, *Ostertagia* and *Trichostrongylus* eggs 10 d after treatment, percent reductions and 95% confidence limits from 9 groups of sheep in experiment 2

	Total eggs			Ostertagia eggs			Trichostrongylus eggs		
Group treatment mg/kg	Mean	% Reduct	95% Limits	Mean	% Reduct	95% Limits	Mean	% Reduct	95% Limits
1. Controls	916	-	-	623	-	-	293	-	-
2. RBZ*+ LEV† 1.3 + 2.3	440	52	23,70	396	36	0,60	44	85	76,91
3. RBZ + LEV 2 + 3.8	376	59	29,76	327	47	9,70	49	83	71,90
4. RBZ + LEV 2.7 + 4.7	354	61	32,78	325	48	8,70	28	90	83,95
5. RBZ + LEV 3.3 + 5.8	314	66	38,81	305	51	11,73	9	97	94,98
6. RBZ + LEV 4 + 6.8	115	88	74,94	112	82	62,91	2	99	98,100
7. RBZ + LEV 4.8 + 8.4	124	86	78,92	116	81	70,88	7	97	96,98
8. RBZ 4	349	62	39,76	227	64	42,77	122	58	34,74
9. LEV 6.8	366	60	29,77	354	44	0,68	15	58	91,97

* RBZ = ricobendazole = albendazole sulphoxide
† LEV = levamisole

TABLE 5

Arithmetic mean of worm egg counts 10 d after treatment, percentge reductions and 95% confidence limits for 5 groups

Group	treatment mg/kg	Mean epg	% reduct	95% Con limits
1	Controls	354	NA	NA
2	RBZ*	245	31	0,80
3	Lev † 7.5	212	40	0,73
4	RBZ + LEV 3.6 + 7.5	45	87	66,95
5	RBZ + LEV 7.2 + 15	34	90	67,97

* RBZ = ricobendazole = albendazole sulphoxide
† LEV = levamisole

worm counts, and standard errors, for *Ostertagia* spp and *T colubriformis* are shown in Table 6. Too few early 4th stage *Ostertagia* and 4th stage *Trichostrongylus* spp were counted to enable separate analysis. Their numbers have been included in the counts of 4th stages and adult worms, respectively. The percentage reductions and 95% confidence limits have been calculated from these data.

Albendazole sulphoxide at 4 mg/kg removed only 7% of adult *Ostertagia* spp but was more effective against the 4th stages of this genus (80%), and against *T colubriformis* was 86% effective. Levamisole at 7.5 mg/kg was highly effective against 4th stage *Ostertagia* spp (90%), but not against adult worms of either *Ostertagia* (47%) or *T colubriformis* (36%). The single dose of mixture comprising 3.6 and 7.5 mg/kg, respectively, of the 2 compounds, removed 68% of adult *Ostertagia* spp and was highly effective against 4th stage parasites and adult *T colubriformis*. A double dose of this mixture was highly effective against all classes of parasite in this population. In the analysis of variance, the interaction term for albendazole sulphoxide and levamisole was not significant ($F_{1,28}=2.21$) at the 5% level of probability.

Discussion

The choice of nematode strains for assessing the efficacy of mixtures of anthelmintics needs to take into account the current widespread prevalence of drug resistance on farms. Clearly, if strains resistant to only one drug in either the benzimidazole or levamisole classes of compound were chosen, a mixture comprising these compounds would be highly effective at the recommended dose rates. Conversely, it could be expected that nematodes resistant to compounds from both classes could predominate on some farms, and the use of mixtures against them would be ineffectual.

Without species differentiation, tests for resistance do not distinguish between apparent multiple resistance due to one or more species being resistant to both classes of anthelmintic, or to an association of different species, each resistant to only one or the other of the compounds. For these reasons, it was considered that strains of *Ostertagia* spp and *T colubriformis* spp with moderate degrees of resistance to benzimidazoles and levamisole, would provide an assessment of the efficacy of anthelmintic mixtures which was likely to reflect the current situation on many farms. Comparing the resistance ratios of 2.8 and 2.9 from the egg-hatch assays with values obtained by Martin et al (1989), the frequency of resistance genes could be estimated to be in the range of 25 to 50% for both strains used in the first experiment.

The first experiment was primarily a test for the presence of synergism between albendazole sulphoxide and levamisole. Data from the 2 dose rates chosen, namely, one-third and two-thirds the recommended dose rate of each compound, provided a sensitive test for synergism because the interaction term in the analysis of variance could be compared with the residual error from all groups with a large number of degrees of freedom. The absence of a significant interaction term was conclusive evidence against the presence of synergism or antagonism (see Figure 1). Therefore, the observed effects of the drug combinations were due simply to the additive effects of each compound. Non-significant tests for the presence of synergism in the subsequent experiments provide further evidence to substantiate this conclusion. These findings are contrary to those reported by Bennet *et al* (1980), who demonstrated synergism between mebendazole

TABLE 6

Arithmetic mean worm counts of *Ostertagia* and *Trichostrongylus* spp, percent reductions and 95% confidence limits from 5 groups of 8 sheep in experiment 3

Group	treatment mg/kg	*Ostertagia* sp Adults worms	Percent reduction	4th stages	Percent reduction	*Trichostrongylus* sp Total worms	Percent reduction
1.	Controls	2817 ± 2070	- -	1602 ± 965	- -	2020 ± 553	- -
2.	RBZ* 4	2610 ± 2036	27 (0,58)	317 ± 281	80 (56,91)	287 ± 247	86 (72,93)
3.	LEV† 7.5	1487 ± 900	47 (0,74)	152 ±186	90 (73,96)	1277 ± 226	36 (19,50)
4.	RBZ + LEV 3.6 + 7.5	900 ± 706	68 (29,85)	47 ± 56	97 (92,99)	95 ± 95	95 (89,98)
5.	RBZ + LEV 7.2 + 15	92 ± 132	96 (89,99)	17 ± 22	98 (97,100)	10 ± 21	100 (98,100)

* RBZ = nicobendezole =albendazole sulphoxide
† LEV = levamisole

and levamisole against a mebendazole resistant strain of *H. contortus*. Unfortunately, the obvious differences in species of nematode, resistance status of the strains, the combinations of drugs and differences in the dose rates used, do not allow strict comparison nor a satisfactory explanation for the different results.

Several other points of interest emerge from the responses to the treatments used in the first experiment. Compared with other groups, the reductions in total egg count were significantly higher in groups 7, 8 and 9 in which the treatments included two-thirds of the recommended dose rate of either anthelmintic. It seems likely that the high efficiency of albendazole sulphoxide against *T colubriformis* contributed to this result. However, comparing the responses of *Ostertagia* spp to the different treatments, it can be seen that the lower dose of levamisole, when combined with either dose rate of albendazole sulphoxide, was relatively ineffective whereas the higher dose of levamisole in either combination produced a reduction in egg count of greater than 90%.

Larvae for the second and third experiments were derived from strains which had been treated with one-third of the recommended dose rates of both compounds. Consequently, adult populations arising from infections of these larvae could be expected to have a higher gene frequency for resistance than those in the first experiment.

The egg-hatch data showed that the resistance ratio for benzimidazoles had increased from about 3 to more than 18, and in Table 4, the efficacy of a recommended dose of albendazole sulphoxide was 64% for *Ostertagia* spp and 58% for *T colubriformis*. Resistance to levamisole in the *Trichostrongylus* population appears to have diminished so that all but the 2 lowest dose combinations, namely, 0.33 and 0.5 of the recommended dose rates, were highly effective in reducing the egg output of these worms.

Comparing the data in experiments 1 and 2 (see Tables 3 and 4), the degree of multiple resistance in the *Ostertagia* population appears to have increased substantially following selection with the one-third doses. The highest percentage reductions were obtained from the 2 highest dose combinations which correspond to 1 and 1.2 times the recommended dose of each drug.

The populations of *Ostertagia* and *Trichostrongylus* spp present in experiment 3 were demonstrably resistant to both benzimidazole and levamisole anthelmintics (see Tables 5 and 6). The dose combinations used in this experiment were equivalent to 0.9 and 1.1 times, respectively, of the recommended doses of albendazole sulphoxide and levamisole and double these quantities.

Both dose rates of the combination produced a high percentage reduction compared to the effects of a single dose of the compounds alone. However, only 69% of adult *Ostertagia* spp was removed by a single dose of both drugs given together, whereas the double dose was highly effective against all categories of parasite tested.

When reviewing the data, for the purpose of choosing an effective dose of albendazole sulphoxide and levamisole for a mixture, the high degree of resistance in the worm populations of experiments 2 and 3, especially for *Ostertagia* spp should be borne in mind.

Clearly, results from all 3 experiments show that to achieve a reduction in worm egg output of at least 90%, the dose rates for the mixture should not be less than the recommended dose rates for the single components. However, dose rates above this level were too few in number in experiment 2 to accurately determine the doses needed for a 90 or 95% reduction in egg output of these strains. The estimates were, respectively, 1.2 and 1.5 times the recommended dose rates, and data from experiment 3 confirmed that dose rates approximately double those recommended were highly effective against strains with substantial degrees of resistance. Of course, such high rates could not be used routinely because of factors such as cost and residues in tissues, but they may have a role in worm control under appropriate circumstances.

Given that there will be nematode populations refractory to treatment with a mixture, it was concluded that the dose rates used in experiment 3 should be tested on farms to determine their efficacy against a number of nematode populations with different degrees of resistance to the benzimidazole and levamisole classes of anthelmintic.

Acknowledgments

We thank Messrs MA Pope, and NL Savory (CSIRO), M McLaren and M Wessels (Young's Animal Health) for their technical assistance and Mr L Carey for his care of the experimental animals. Young s Animal Health Pty Ltd provided the funds and anthelmintics for the conduct of this work.

References

Anderson N (1968) *Bovine Ostertagiasis*, PhD thesis, University of Glasgow, Scotland

Anderson N (1972) *Aust J Agric Res* **23**: 1113

Anderson N, Martin PJ and Jarrett RG (1988) *Aust Vet J* **65**: 62

Bennet EM, Behm C, Bryant C, and Chevis RAF (1980) *Vet Parasitol* **7**: 207

Clark CJ and Turton JA (1973) *Exp Parasitol* **34**: 69

Curtis CF (1985) *Bull Entomol Res* **75**: 259

Douvres FW (1957) *Proc Helminthol Soc Wash* **24**: 4

Le Jambre LF (1976) *Vet Parasitol* **2**: 385

Mani GS (1985) *Genetics* **109**: 761

Martin PJ, Anderson N, Jarrett RG, Brown TH and Ford GE (1982) *Aust Vet J* **58**: 185

Martin PJ, Anderson N, Lwin T, Nelson G and Morgan TE (1984) *Int J Parasitol* **14**: 177

Martin PJ, Anderson N and Jarrett RG (1989) *Aust Vet J* **66**: 23b

Moskey HE and Harwood PD (1941) *Am J Vet Res* **2**: 55

Snedecor GW and Cochran WG (1967) *Statistical Methods*, 6th edn, Iowa State University Press, Ames, Iowa

Waller PJ, Dobson RJ, Donald AD, Griffiths DA and Smith EF (1985) *Int J Parasitol* **15**: 669

(Accepted for publication 1 November 1990)

Appendix 1

Confidence intervals for percentage reductions. The sample mean and variance of the worm-egg counts from a control and a treated group of n sheep are, respectively, X_c, s_c^2 and $\bar{X}_t$, s_t^2.

The percentage reduction due to the treatment is then estimated by $R=100(1-X_t/X_c)$.

As a first step, 95% confidence intervals are obtained for the log of the ratio of means as

$$\log(\bar{X}_t) - \log(\bar{X}_c) \pm 2\sqrt{\mathrm{Var}\{\log(\bar{X}_t)\} + \mathrm{Var}\{\log(\bar{X}_c)\}}$$

Now, $\mathrm{Var}(\bar{X}_t) = s_t^2/n$ and, by a Taylor series expansion which is valid when $s^2/(n\bar{X}_t^2)$ is small (<0.2 say),

$$\mathrm{Var}\{\log(\bar{X}_t)\} \cong s_t^2/(n\bar{X}_t^2),$$

with a similar formula for $\mathrm{Var}\{\log(\bar{X}_c)\}$ (see, for example, Snedecor and Cochran, 1967). Thus a 95% confidence interval for the percentage reduction is

$$100\,[1-(\bar{X}_t/\bar{X}_c)\exp\{\pm 2\sqrt{(s_t^2/\bar{X}_t^2 + s_c^2/\bar{X}_c^2)/n}\}]$$

This is to be preferred to the formula of Clark and Turton (1973) which was

$$100\,[1-(\bar{X}_t/\bar{X}_c)\{1 \pm 2\sqrt{(s_t^2/\bar{X}_t^2 + s_c^2/\bar{X}_c^2)/n}\}],$$

and which may give nonsense values when the variance is large.