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AIMS AND FINDINGS OF COOPERATIVE EXPERIMENT

In its preliminary survey of the problem, both in its clinical and its practical aspects, it appeared to the Committee that the discrepancies in the findings of different workers were due, at least in part, to these having had different aims, and having tested the efficacy of the drug by different criteria of success. It will appear from the results consistently found ~~that~~ at the four centres carrying out the Committee's own series of experiments, that this circumstance, in conjunction with frequent differences in the level of dosage tested, is capable of explaining very wide discrepancies without calling for explanation upon the ordinarily differences existing between different batches of experimental material, such as, in our problem, differences in age of lambs tested, or differences in breed and previous treatment.

There are three observable characteristics of treated or untreated sheep by which the efficacy of treatment from different standpoints can be assessed.

(a) Egg counts in faecal matter, the technique for the evaluation of which is fully set out in Section ( ). This observation has the great advantage that it can be carried out on living animals, and therefore is of value not only after a period of treatment, as a means of gauging its efficacy, but also as a preliminary observation before treatment has been differentiated as a means of measuring the degree of infestation with which each lamb enters the experiment. It is also capable of relatively great quantitative precision

The diverse species of parasitic worms are not ordinarily differentiated in the egg counts, although by further technical improvements this may be later possible. The counts taken at the four cooperating centres distinguish the eggs of Strongyloides and Nematodirus, and a third group comprising the total of the eggs counted from other species.

(b) The worm counts: After slaughter direct counts, or sampling estimates, may be made of the numbers of adult worms, distinguished at least so far as genera, found in the fourth stomach, or abomasum or in the small or large intestine. Both qualitatively and quantitatively such counts afford a better assessment of intensity of infestation than can be afforded by egg counts. They can, however, only be taken on slaughtered sheep and cannot, for this reason, be used to assess the initial degree of infestation.

(c) Live weight: The weights of sheep were taken initially and at intervals throughout the experiment in each experimental flock. From the point of view of practical application in animal husbandry, the progress of the animal in growth as measured by live weight is of importance superior to that obtainable by any purely clinical observation, for it affords a measure of the net effect of treatment on the commercial value of the animal, whether destined for immediate slaughter or for breeding. Since, apart from the effect of drugs upon parasitic species, the drugs may be presumed to have, and in fact show evidence of having, direct, though perhaps temporary, effects upon the growth of the lambs, it is essential that the net consequences of treatment through all chains of causation, explored or unexplored, should be assessed in determining its practical value

With these criteria in view, it was decided to test the net response in live weight on an "outside flock" at each centre, kept in normal farming conditions, including exposure to reinfection, and maintained under observation for 15 weeks following treatment, on the supposition that the effects visible after this period would approximate to the final effects of the drug. Initial egg counts were taken on these sheep, and later egg counts are now available for some of these flocks, though this was not required by the experiment. These sheep were not slaughtered for worm counts. Six different outside flocks have been recorded.

In addition an "inside flock" was established at each of the cooperating centres, intended for slaughter approximately 5 weeks after treatment. Since these were to supply worm counts, it was necessary to avoid reinfestation, and consequently to treat the flock rather as experimental than as farm animals. The interest of the inside flocks centres in the worm counts and associated final egg counts in relation to the initial egg counts and treatment of the animals. Weights were also available from the inside flocks.

Appendix (see Table 3)

Apart from additional animals added at certain centres, each flock outside and inside, comprised 28 lambs, four each being assigned to the dosage levels of 0, 5, 10, 20, 30, 40, 50 g. phenothiazine. Of the four lambs treated at any level, two received the dose in the form of tablets, two in the form of a drench. The wide range of the dosages chosen is due to previous published work having indicated that the egg count might be responsive to quite small doses, without appreciable benefit in weight, while from other work it appeared that the growth rate might be benefited by larger doses.

Summarised outcome of trials.

The effect of phenothiazine in reducing total egg density in the faeces.

The density with which the eggs of parasites are found in the faeces is obviously closely connected with the extent to which infested sheep contribute through the contamination of pastures to the infestation of others. The importance of such counts would be increased if the eggs of different species could be distinguished. However, since the total egg count is widely used, it is important to throw all possible light on the interpretation of this means of measuring disinfestation. (see appendix, Table 3)

The total egg count varied greatly from sheep to sheep within each flock. Since, with sheep treated alike, this variation was remarkably parallel in the final and in the initial egg counts, it was possible greatly to refine the comparison between treatments by using within each flock the records of sheep treated alike to indicate what ratio in the final egg count is to be expected for a given ratio in the initial egg count. Proportional expectation may thus be obtained for each sheep, or each group of sheep, with which the observed final egg counts can be compared. Taking the ratio of final egg count to expectation to be 100 for the untreated sheep of each inside flock, the following values were obtained for the percentage final egg count observed at each dosage level.

Table 1.

Effect of different doses in diminishing total egg count.

Dosage grams per sheep	Edinburgh	Winches Farm	Weybridge	Cambridge	Average
0	100	100	100	100	100
5	35	42	42	52	43
10	17	59	34	15	31
20	17	9	48	49	31
30	17	33	24	33	27
40	9	13	15	82	30
50	9	18	15	51	23

It will be seen that all centres, and at all doses (twenty-four comparisons in all) the final egg count is diminished in comparison with the untreated sheep. Secondly, the greater part of the diminution has been brought about by the first 5 g. of the dose. Evidently extremely small doses are effective in diminishing the egg count. Beyond this level the diminution is comparatively small, and in most flocks too irregular to be detected on the values for only 4 sheep. On the average of four flocks, however, the 50 gram counts give the lowest figure, though beyond 10 grams the changes are irregular.

It will appear from the worm counts (appendix Table 4) that different species differ greatly in susceptibility to the drug. The most susceptible species, Haemonchus, is believed to contribute largely to the egg count, while some of the less susceptible may contribute relatively few eggs. This consideration may be considered sufficient to explain the high apparent efficacy of small doses, as also the fact that the response as measured by egg count is apparently ~~quicker~~ greater in some flocks than in others, at Edinburgh, for example, than at Cambridge. The response at Edinburgh was so regular that an attempt was made to compare the actual counts with a series of expectations based on the common

assumption of dosage mortality curves that the logarithmic dosage is a linear function of a normal deviate, the probability of exceeding which is the chance of survival of the organism. That such a relationship is capable of formulating the type of response observed is shown in the following table.

Table 2

Final egg count at Edinburgh

Dosage	Expected	Observed	Difference
0	620.65	620	-0.65
5	299.04	335	+35.96
10	222.71	168	-54.71
20	77.38	79	+ 1.62
30	118.53	154	+35.47
40	74.57	61	-13.57
50	53.12	49	- 4.12
Total	1466.00	1466	0.00

It should be noted that the comparison of 20 g. is based on only 3 sheep, so that the total egg counts, expected and observed, are both reduced. Obviously very high doses would be needed to decrease the egg count in a much higher ratio.

Worm counts

The actual worm counts from individual sheep are set out in Table 4 (Appendix). No satisfactory statistical summary of such material seems possible. The frequencies for any one species of worm differ so greatly from sheep to sheep that no uniformity is to be expected from totals or averages. Moreover the measure of initial infestation supplied by the egg count refers to a mixture of many species, and the attempt made with respect to Nematodirus to improve the comparison by this means failed through the comparatively small numbers of eggs counted from this species.

both

Surveying the data with respect to frequency and degree of infestation, it is apparent that in all flocks Haemonchus is more completely eliminated by light doses than any other species. The two other parasites of the abomasum , Ostertagia and Trichostrongylus axei are also susceptible to doses up to 20 grams, at which point half or more of the sheep are free from infestation. The intestinal parasites may be classified in order of susceptibility as follows:-

Class	Small intestine	Large intestine
B	Cooperia, Trichostrongylus (Spp), Bunostomum	
C		Chabertia, Oesophagostomum
D	Strongyloides	Trichuris
E	Moniezia, Nematodirus	

In class E the tape worm Moniezia appears to be distinctly more abundant among sheep heavily dosed than among controls or sheep lightly dosed. The same may be true of Nematodirus, the frequencies recorded for which are, however, expectionally irregular.

In class D there is some apparent increase from 0 to 20 or 30 grams, with a decrease for higher doses. It appears possible that owing to the removal of competing species, the parasites of class E are able to maintain their infestation of lambs from whibh, without treatment, they would have been to a greater or less extent eliminated, and that doses of 20 or 30 grams confer a similar benefit on the members of class D. Of the 12 species mentioned, there can be little doubt that of the first eight surviving adult parasites are appreciably diminished, and at the higher doses some progeess has been made towards disinfestation from the two parasites of class D also.

Effect on Live Weight

For a decision as to the value in farming practice of treatment with phenothiazine, reliance was placed on the weight increases over a period of fifteen weeks of the outside flocks. Of these there were six, two tested at Edinburgh and Winches Farm respectively, while four more were obtained by Dr Taylor at Weybridge. These will be designated by the names of their owners. - Appendix see Table 5

The final weight recorded for each sheep is, as might be expected, strongly associated with the weight at which it enters the experiment. In other material tested for shorter periods it has also been found to be sufficiently closely associated with the initial egg count for the use of this factor also in calculating expected final weights to give added precision to the comparisons. In the comparisons reported here therefore account has been taken of both factors, although the initial egg counts contributed little to the final precision, as should perhaps have been expected from the fact that for fifteen weeks the majority of the sheep tested had been more or less thoroughly disinfected. The adjustments for initial egg counts were therefore small, and make little difference to the final results. This point is noted in view of the reasonable criticism that any allowance for initial infestation is, if the treatment is successful, likely to be an overestimate at the large doses and penalize the lightly infested sheep at these levels, while at low dosage levels the more heavily infested sheep will be put at an apparent disadvantage. Since the sheep were assigned at random to treatments, no bias is introduced into the results. But very much more elaborate calculation would have been needed to do just

justice to the possible advantage given by initial egg counts in improvingthe precision of weight comparisons. With this defect, the comparisons available give a standard error of only 4½ lbs for the individual sheep, which is a satisfactory level for practical experiments in animal husbandry. For the average of six flocks at any one dosage level the standard error is just less than one pound in live weight. Table 6 gives the adjusted final mean live weight in comparison with that of the controls for each of the six flocks and for the average of all at each dosage level.

Table 6

Increases in live weight compared with untreated lambs, in lbs per sheep.

Dosage in g.	Winches Farm	Edinburgh	Cook	Horne	Rattenbury	Scott	Average
5	2.51	-1.07	3·99	1.03	8.07	1.04	0.92
10	-1.70	-4.68	3·63	-0.29	10.74	-1.11	-0.16
20	3.60	-0.28	0·54	5.89	8.69	4.43	3.55
30	1.35	4.65	4·31	2.13	9.78	1.28	3.65
40	7.78	1.25	1·65	5.35	7.92	1.54	4.17
50	6.08	2.90	1·57	5.49	12.34	4.68	5.36

The individual figures based on only 3 or 4 sheep are somewhat irregular, and it should be noticed that,as each comparison is made with the control sheep, all the values given for any flock will be raised or lowered alike according to the performance of these. Over the 6 flocks it may be observed that in every case the groups receiving 30, 40 or 50 grams invariably show an improvement compared with the controls, and on the average of the 6 flocks the gains in live weight ascribable to treatment are seen to be large, and to increase rather regularly with dosage.

In view of the possibility that these live weight data should

favour particular dosage levels, each flock was tested for linearity of response in live weight to weight of drug administred. In no case were the departures from proportionality statistically significant. The mean response expressed in pounds live weight per 10g unit of drug are indeed more concomdant than could have been expected from the variations of individual sheep. These are:

Winches Farm	Edinburgh	Cook	Horne	Rattenbury	Scott
1.38	0.88	1.04	1.15	1.19	0.73

The general average is 1.057 lbs live weight per 10 gs phenothiazine, or approximately 48 times the weight of the drug, nearly independently of dosage within the range explored. Since the different flocks differed undoubtedly both in degree and kind of infestation the concordance of their average responses is an indication that simila responses could have been obtained in practical conditions from a very large number of commercial flocks.