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A MODIFICATION OF STANDARD PROCEDURES FOR EVALUATING THE RELATIVE EFFICACY OF ANTHELMINTICS

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Although only one of the links in the chain of any system of control of helminthiasis of domestic animals, is the destruction and elimination of intestinal parasites, it is essential that the relative efficacy of available anthelmintics should be known with reasonable accuracy.

Apart from the results of mere clinical trials which are of little real value, three methods are in use to determine the efficacy of a drug or combination of drugs: the faecal egg count method in the live animal, the critical technique of Hall & Foster (1918) and the controlled test of Moskey & Harwood (1941).

The faecal egg count method is based upon a count of the number of eggs per gram of faeces before and after the administration of an anthelmintic. Without entering into any discussion on the merits and demerits of this technique which have been adequately covered by Gordon (1950), it is necessary merely to point out that it is generally accepted today that the number of ova excreted in the faeces is not necessarily an index of the worm burden of the host. Therefore, it is believed that this method should be regarded as a rough screening procedure to indicate anthelmintic activity, before embarking upon more accurate but more time-consuming, laborious and expensive slaughter tests (Reinecke & Rossiter, 1962).

Hall & Foster's (1918) critical test has as its basis the treatment of worm infested animals, and the collection of the entire faecal output for four days after treatment for identification and counting of all worms excreted. Thereupon the animal is slaughtered and all residual worms in the alimentary tract are counted. The efficacy of the drug is then expressed as a percentage of excreted worms to the total number counted. The chief disadvantage of the method is the time and highly skilled labour involved in the examination of large masses of excreta and intestinal contents, particularly when no worms at all may be found in the faeces on the third and fourth days. The chief advantage is that each animal acts as its own control so that the number of animals sacrificed to obtain a statistically accurate and significant evaluation of drug efficacy is reduced to a minimum.

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The controlled test of Moskey & Harwood (1941) involves the artificial infestation of susceptible animals reared under worm-free conditions. When the worms reach maturity one half of the experimental animals selected at random is treated with the drug under test while the other half is retained as controls. Two weeks after treatment, during which time the entire experimental group is maintained under worm-free conditions, all the animals are slaughtered and the number of worms in each counted. The number of worms recovered from the untreated controls is taken as the probable number of worms in the treated animals at the time of treatment; the number of worms in the treated animals constitutes those unaffected by the drug. From these figures the percentage efficacy of the drug is calculated. It is obvious that to obtain an accurate estimate of drug efficacy by this method more experimental animals are required than is the case with critical trials.

In the present investigations, an attempt has been made to take advantage of the merits of each of the above two slaughter methods in developing simplified standard techniques as a basis for evaluating the relative anthelmintic efficacy of remedies.

As anthelmintics three drugs were selected: Thiabendazole (Merck, Sharp & Dohme), Methyridine (Imperial Chemical Industries) and a Phenothiazine-coroxon mixture (Cooper & Nephews) referred to in the text by its trade name Coopex.

A.—Critical Tests (Modified Hall & Foster method)

Material and methods

Naturally infested sheep and worm-free lambs were dosed with 20,000 to 200,000 infective larvae of the species of worm under investigation. When it was judged that the worms had reached maturity either by the appearance of eggs in the faeces or by an increase in the number of eggs per gram of faeces (e.p.g.) the animals were treated with the particular anthelmintic.

After treatment a faecal collecting bag was attached and replaced every day up to the time of slaughter to collect all faeces for examination.

After slaughter, on opening the abdominal cavity, tight double ligatures about an inch apart, were tied round the alimentary tract at the following points:—

(1) The abomasum at both the fundus and pylorus.

(2) The duodenum at the duodeno-colic ligament at the commencement of the jejunum.

(3) The ileum at the ileo-caecal valve.

After stripping the mesentery the gut was severed between the double ligatures to yield four isolated portions of the gastro-intestinal tract which could be handled separately.

Each portion of the gut was then opened and the ingesta washed through sieves (100 and 200 mesh to the linear inch) the sievings finally being washed into labelled jars to which formalin was added to a final concentration of 10 per cent. Each portion of gut, cut into pieces 3–4 inches long, was placed in separate jars, digested with pepsin and sieved (Reinecke, 1961).

Faeces from the faecal collecting bag from each sheep were pooled, and soaked in water for a few hours to soften them. After breaking up the pellets by hand they were sieved and the sievings collected in a manner similar to the ingesta.

For the purpose of counting the worms in each separate pool, water was added to the sievings until a thin suspension was formed and the volume adjusted to the nearest litre, this being 2 litres in most cases. Aliquots from each diluted pool were collected in the following manner: a glass pipette with a wide mouth, such as a 20 ml graduated pipette with the tip cut off, was used to blow air vigorously through the fluid to produce a uniform suspension. The stream of air was discontinued and immediately a volume of fluid transferred to a suitable calibrated container. The procedure was repeated until a volume not less than one tenth of the total volume had been collected. From each pool three such aliquots were collected.

For counting and identifying the worms each aliquot was stained with iodine, a few ml pipetted into a square glass counting chamber (3 by 3 by $\frac{1}{2}$ in) and decolorized with sodium thiosulphate (Whitlock, 1948). The brown-stained worms were readily seen and could be counted under a dissecting microscope. When necessary individual worms were removed for examination under higher magnification. The process was repeated until all the worms in three aliquots had been counted. If the number of worms in the three aliquots varied by more than 10 per cent from the mean, further aliquots were counted. From these counts the total number of worms in each separate pool might be calculated. If the number of worms in the first aliquot was 100 or less, indicating a grand total of less than 1,000, the entire pool was examined. Usually this was the case with the digested gut and with adult *Oesophagostomum columbianum*.

For calculating the anthelmintic efficacy of a drug due attention was paid to the normal habitat of the various worm species. *Haemonchus contortus* and *Ostertagia circumcincta* normally parasitize the abomasum though small numbers may be found in the duodenum but not distal to it. Therefore, any worms of these species found distal to the duodenum were regarded as expelled worms. Similarly, species which normally infest the small intestine were regarded as expelled when found in the caecum, and caecal or colonic species as expelled only when found in the contents of the faecal bag. From the above the percentage efficacy of a drug may be calculated from the simple formula—

$$\text{Percentage efficacy} = \frac{\text{No. of expelled worms}}{\text{Total No. of worms}} \times 100.$$

To illustrate the method the results of a critical test on a single sheep (No. 590) are given in Table 1.

TABLE 1.—*The results of a critical test on a sheep treated with* 200 *mg/Kg Methyridine subcutaneously and slaughtered* 36 *hours later*

Species	Stage of development	Worms recovered					Efficacy %
		Abo.	Duo.	Je. Il.	C.C.	F.	
H. cont	*A.	10	0	x 0	0	67	87·0
Oster	A.	1,100	0	x 0	0	170	13·4
Trich	†4th	0	230	110	x 0	0	0
	A.	0	15	200	x 10	2,892	93·1
O. col	4th.	0	0	20	140	x 170	51·5
	A.	—	—	—	0	x 110	100
T. parvi	A.	—	—	—	0	x 5	100

KEY

H. cont. = *Haemonchus contortus*
Oster. = *Ostertagia circumcincta*
Trich. = *Trichostrongylus colubriformis*
O. col. = *Oesophagostomum columbianum*
T. parvi. = *Trichuris parvispiculum*
Abo. = Abomasum
Duo. = Duodenum
Je. Il. = Jejunum and Ileum
C.C. = Caecum and Colon
F. = Faeces collected in bags
†4th = Fourth stage larvae
*A. = Adult worms
x Worms to the right of this line are regarded as expelled worms

Results

This example of a single autopsy is merely given to illustrate the method. Although some species were well represented, others e.g. *H. contortus* were present in small numbers and no conclusions can be drawn until more animals have been examined.

The need for short term critical slaughter trials has been suggested by the extremely rapid action of some anthelmintics such as Methyridine which was known to cause the expulsion of worms in 16–24 hours and Thiabendazole in less than 48 hours.

An illustration of the rapid action of Thiabendazole is given in Table 2.

TABLE 2.—*The rapidity of action of Thiabendazole dosed at* 50 *mg/Kg*

Sheep No.	Species	Worms recovered				
		(*a*) in faeces after dosing on day				(*b*) Post mortem
		1	2	3	4	
56	*Trichostrongylus* spp.....	11,206	259	0	1	0
586	*Oesophagostomum columbianum*	181	4	0	0	0

Comment

It is obvious that both Methyridine and Thiabendazole act very rapidly (cf. Tables 1 and 2). Affected worms are either in faecal bags or distal to their normal habitat within 48 hours and can be regarded as expelled worms. No useful object could be served by collecting negative faeces after two days when the drug had already had its effect.

At this stage it was decided to apply this modification of the critical test to a determination of the relative efficacy of the three selected drugs against *H. contortus*, *O. circumcincta*, *Trichostrongylus* spp. and *O. columbianum*. The results are shown in tabular form in Tables 3 to 6.

(*a*) *Haemonchus contortus*

TABLE 3.—*Modified critical tests*: Haemonchus contortus

Sheep No.	Interval between dosing and slaughter: in hours	Stage of development	Worms recovered					Efficacy %
			In normal habitat		Distal to normal habitat			
			Abo.	Duo.	Je. Il.	C.C.	F.	
	Thiabendazole 50 mg/Kg *per os*							
62	6	A.	4,246	922	0	312	0	22·5
79	12	A.	41	2	0	0	0	0
84	12	A.	472	0	120	0	15	22·0
83	14	A.	132	11	73	735	200	87·5
78	14	A.	2,300	151	253	75	0	12·0
69	16	4th	1,466	143	48	90	10	8·0
		A.	2,234	113	3	40	0	2·0
81	16	A.	280	31	130	165	0	48·6
75	16	A.	60	21	20	30	225	77·0
85	22	A.	0	0	15	0	40	100·0
5	22	A.	0	0	30	0	40	100·0
7	22	A.	80	0	0	0	120	60·0
*14	96	A.	0	0	0	0	3	100·0
	Methyridine 200 mg/Kg subcutaneously							
984	24	A.	0	0	0	0	50	100·0
90	36	A.	10	0	0	0	67	87·0
87	46	3rd.	0	300	0	0	0	0
	Coopex 200 mg/Kg *per os*							
575	24	3rd.	590	0	0	0	0	0
		4th.	885	0	0	0	0	0
		A.	200	0	0	0	0	0
50	36	A.	17	0	0	10	200	92·5
36	48	4th.	2,340	22	0	0	0	0
		A.	0	0	0	0	240	100·0
91	48	4th.	136	0	20	0	0	12·8

* This sheep showed a *Haemonchus contortus* egg count of 30,000 eggs per gram (e.p.g.) on the day of dosing. See text.

KEY

Stages of development: 3rd = Third stage larvae.
4th = Fourth stage larvae.
A. = Adult.

Results

On completion of the worm counts a striking point in regard to *H. contortus* emerged. If reference is made to Table 3, sheep 14 was shown to have an *H. contortus* egg count of 30,000 e.p.g. on the day of treatment. At the time it was debilitated, weak, and anaemic with a pronounced submandibular oedema; for 36 hours after dosing only a few pellets were defaecated. Yet at the time of slaughter at the ninety-sixth hour no wireworms were found in either the abomasum or intestines and only three in the faeces collected.

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In an attempt to explain the disappearance of the parasites a number of fresh *H. contortus* was incubated at 38°C in petri dishes in an artificial digestion medium consisting of *Trypsin 0·4 per cent, †Bile salts 1·6 per cent, $NaHCO_3$ 1·0 per cent, NaCl 0·8 per cent adjusted to pH 8·0. Within two hours some of the worms were partially digested and after six hours many were digested to a point where only the outer cuticle was still recognizable. These fragments would have escaped detection in a critical test. On the other hand *O. circumcincta* was not as rapidly digested. This indicated that the method is unreliable for *H. contortus*, a point to be taken into consideration when evaluating the results of the critical tests.

It is concluded that Thiabendazole is highly effective but not earlier than 22 hours after treatment at a dosage of 50 mg/Kg. Methyridine at the standard dose of 200 mg/Kg is somewhat inconsistent, but in view of the explanation given above the percentage efficacy may be higher than that indicated. Coopex at the standard dosage of 200 mg/Kg is highly effective after 36 hours.

All three drugs are ineffective against the immature forms of the parasite. This finding may be due to a defect of this method in regard to *H. contortus* as will be seen later (cf. results of modified controlled tests).

(*b*) *Ostertagia circumcincta*

Results (Table 4)

Thiabendazole at a dosage rate of 25 mg/Kg was highly effective in one out of two sheep in a period of 43 hours. At the standard dosage rate of 50 mg/Kg the efficiency up to 24 hours was variable, but by 97 hours the lethal effect was pronounced. When the dose was further increased to 75 mg/Kg it was variably effective up to 18 hours but by 43 hours was highly effective. It would appear that the drug is effective at the standard dosage rate but is somewhat slow-acting.

The efficacy of Methyridine and Coopex was low and variable at the standard dosage rate of 200 mg/Kg after 48 hours. Neither drug appeared to exert any lethal effect upon immature stages of the parasite.

Although the number of well-preserved worms recovered distal to the duodenum and from the faeces bags confirmed the observation that this method is more applicable to trials on *O. circumcincta* than on *H. contortus*, their numbers were not large enough to justify the conclusion that this method was entirely satisfactory. Some doubt still existed whether they had not also been digested after being killed by the drug, even if the digestion was of a lower order than was the case with *H. contortus*.

(*c*) *Trichostrongylus spp.*

Results (Table 5)

Thiabendazole was highly effective after 43 hours even at the lowest dosage rate of 25 mg/Kg. This is supported by the highly effective results obtained with higher doses within a shorter period; but it must be emphasised that the full effect of treatment cannot be expected in less than 24 hours. A point of particular importance is that in one sheep (No. 89) the drug was 86·2 per cent effective against fourth stage larvae.

Methyridine was as effective as Thiabendazole but was of no value against the immature forms.

The efficacy of Coopex at a dosage rate of 200 mg/Kg was disappointingly low against both mature and immature worms.

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TABLE 4.—*Modified critical tests*: Ostertagia circumcincta

Sheep No.	Interval between dosing and slaughter: in hours	Stage of development	Worms recovered					Efficacy %
			In normal habitat		Distal to normal habitat			
			Abo.	Duo.	Je. Il.	C.C.	F.	
	Thiabendazole 25 mg/Kg *per os*							
76	43	A.	125	27	60	540	896	90·1
79	43	A.	306	0	0	50	20	18·6
	Thiabendazole 50 mg/Kg *per os*							
77	16	A.	180	11	150	300	180	76·7
86	16	A.	890	7	45	0	60	10·6
82	18	A.	7,670	185	190	60	0	3·1
89	24	A.	92	8	80	755	60	90·0
80	24	A.	3,170	101	210	0	0	6·0
	Thiabendazole 75 mg/Kg *per os*							
70	16	A.	5,950	340	740	560	120	15·0
81	16	A.	175	0	60	30	80	46·0
977	18	A.	1,002	56	220	240	90	34·0
582	43	A.	0	1	0	40	195	99·6
85	43	A.	2	11	0	0	35	72·9
	Methyridine 200 mg/Kg subcutaneously							
580	24	A.	153	7	0	80	480	78·0
190	26	A.	116	19	8	0	115	47·3
90	36	A.	1,110	0	0	0	170	13·4
87	46	3rd.	0	400	0	0	0	0
		A.	675	0	1	0	0	0
982	48	4th.	20	0	0	0	0	0
		A.	718	8	0	0	0	0
	Coopex 200 mg/Kg *per os*							
981	24	A.	285	0	0	0	100	26·0
75	24	3rd.	25	0	0	0	0	0
		4th.	5,314	20	0	0	0	0
		A.	2,625	31	40	10	0	1·8
83	24	4th.	52	29	0	0	0	0
		A.	2,132	167	0	75	30	4·4
91	48	4th.	1,624	30	0	0	0	0
		A.	210	20	0	0	160	41·0

If Table 5 is examined it will be noted that large numbers of well-preserved worms were often found in faecal bags e.g. 7,774 in sheep No. 576 and 11,466 in sheep No. 56. This gave clear evidence of the suitability of the critical trial for adult worms of this genus.

(*d*) *Oesophagostomum columbianum*

Results (Table 6)

Thiabendazole dosed at a rate of 25 mg/Kg was found to be fairly effective at the forty-third hour against adults but was inert against immature worms. At twice or three times the dose the drug was highly effective as early as the sixteenth or eighteenth hour against adult worms and at least in one sheep showed some lethal effect against larval stages.

TABLE 5.—*Modified critical tests*: Trichostrongylus *spp.*

Sheep No.	Interval between dosing and slaughter: in hours	Stage of development	Worms recovered: In normal habitat: Abo.	Duo.	Je. Il.	Distal to normal habitat: C.C.	F.	Efficacy %
		Thiabendazole 25 mg/Kg *per os*						
576	43	A.	0	7	15	0	7,774	99·7
579	43	A.	0	0	10	10	1,230	99·2
		Thiabendazole 50 mg/Kg *per os*						
*581	8	A.	1	59	1,409	1,399	49	49·0
79	12	A.	110	9	80	2,640	1,155	95·0
84	12	A.	118	0	15	425	270	83·9
83	14	A.	13	0	0	165	140	95·9
78	14	A.	20	0	0	105	15	85·7
69	16	A.	0	0	0	260	0	100·0
81	16	A.	0	0	10	300	0	96·8
75	16	A.	1	0	0	150	105	99·9
86	16	A.	230	1	30	0	0	0
89	24	4th.	0	4	0	25	0	86·2
		A.	8	0	2	1,840	1,409	99·7
609	36	A.	1	0	30	7	300	90·8
96	48	A.	0	0	0	60	1,200	100·0
577	90	A.	0	0	0	0	606	100·0
584	90	A.	0	0	0	0	360	100·0
586	90	A.	0	0	0	0	655	100·0
35	97	A.	0	0	0	0	4,037	100·0
56	97	A.	0	0	0	0	11,466	100·0
		Thiabendazole 75 mg/Kg						
70	16	A.	0	0	130	400	0	75·5
582	43	A.	0	0	0	0	2,874	100·0
585	43	A.	0	7	1	0	3,966	99·8
		Methyridine 200 mg/Kg subcutaneously						
78	16	A.	5	0	0	202	2,140	99·8
84	24	A.	2	3	0	1	150	96·8
80	24	A.	30	7	315	195	3,930	91·6
90	26	4th.	1	6	2	0	0	0
		A.	28	16	96	740	218	87·3
590	36	4th.	0	230	110	0	0	0
		A.	0	15	200	10	2,892	93·1
87	46	3rd.	0	900	0	0	0	0
		A.	0	0	0	0	175	100·0
982	48	4th.	16	0	0	0	0	0
		A.	842	2,076	6,370	152	60	2·2
		Coopex 200 mg/Kg *per os*						
981	24	A.	650	4,080	21,258	151	1,200	4·9
575	24	4th.	885	30	80	0	0	0
		A.	5,925	417	2,360	60	260	3·5
583	24	4th.	2	107	15	0	0	0
		A.	22	1,095	1,285	690	1,320	45·6
50	36	4th.	0	229	0	10	0	4·2
		A.	2	3	6	0	0	0
400	48	4th.	14	0	130	0	160	52·6
		A.	142	10	100	0	520	67·4
36	48	A.	20	10	0	0	120	80·0
91	48	4th.	0	175	36	0	0	0
		A.	1,210	745	1,088	240	5,640	65·9

* Sheep moribund—did not defaecate.

TABLE 6.—*Modified critical tests*: Oesophagostomum columbianum

Sheep No.	Interval between dosing and slaughter: hours	Stage of development	Worms recovered		Efficacy %
			In normal habitat	In faecal bags	
Thiabendazole 25 mg/Kg *per os*					
576	43	4th	20	0	0
		A.	40	277	87·4
579	43	4th.	9	0	0
		A.	31	105	77·2
Thiabendazole 50 mg/Kg *per os*					
*62	6	4th.	63	0	0
		A.	621	0	0
*581	8	A.	46	0	0
79	12	A.	300	180	37·5
84	12	A.	100	300	75·0
78	14	A.	120	345	74·2
69	16	A.	190	0	0
81	16	A.	375	405	51·9
75	16	A.	135	390	74·3
77	16	A.	60	20	25·0
86	16	A.	0	60	100·0
82	18	A.	45	360	88·9
89	24	4th.	195	80	29·1
		A.	80	5	5·9
96	48	A.	0	100	100·0
577	90	4th.	5	0	0
		A.	0	170	100·0
586	90	A.	0	185	100·0
Thiabendazole 75 mg/Kg *per os*					
977	18	A.	60	240	80·0
582	43	A.	0	49	100·0
585	43	A.	0	92	100·0
Methyridine 200 mg/Kg subcutaneously					
78	16	4th.	27	0	0
		A.	0	160	100·0
84	24	A.	0	50	100·0
80	24	4th.	695	150	17·8
		A.	0	60	100·0
90	26	A.	0	128	100·0
590	36	4th.	160	170	51·5
		A.	0	110	100·0
87	46	3rd.	200	0	0
		4th.	892	0	0
		A.	0	25	100·0
Coopex 200 mg/Kg *per os*					
575	24	3rd.	32	0	0
		4th.	2,540	800	24·0
583	24	4th.	375	40	9·6
		A.	45	50	52·6
50	36	3rd.	1	0	0
		4th.	0	20	100·0
		A.	38	102	72·9
400	48	4th.	15	0	0
		A.	0	40	100·0
36	48	A.	40	0	0
91	48	3rd.	5	0	0
		4th.	1,570	0	0
		A.	90	80	47·1

* Both sheep were moribund and *in extremis* and did not defaecate from the time of dosing to death.

Methyridine at 200 mg/Kg was 100 per cent effective against adults as early as the sixteenth hour and showed a variable but significant effect against immature worms.

Coopex at 200 mg/Kg in one instance was 100 per cent effective against immature worms but in four other sheep had no effect. Against adult worms the efficacy was variable and disappointingly low.

The critical test was well suited to anthelmintic trials on adult *O. columbianum*.

Conclusion

These experiments have proved that Thiabendazole is the most consistently effective anthelmintic. At the standard dosage rate a high lethal effect may be apparent in individual animals in as short a period as 16 hours, yet the full effect may be delayed for as long as 48 hours. Methyridine is highly effective against adult intestinal worms which may be destroyed in as short a period as 16 hours but its effect upon abomasal parasites is erratic and variable. Coopex appears to be inconsistent in its effect and the percentage efficacy is relatively low.

It would appear that the modified critical method is a simplified, time-saving and effective technique for determining the percentage efficacy of anthelmintics, particularly in the case of drugs with a rapid lethal action. It is not suitable for trials against species of worms subject to trypsin digestion in the intestines such as *H. contortus* or immature worms. Therefore controlled tests on these worms were resorted to.

B.—Controlled Tests (Modified Moskey & Harwood method)

Immature worms

Materials and methods

Forty lightly infested, yearling sheep were divided into three groups, each group being dosed with infective larvae as follows:—

Group 1: 3 sheep each receiving 100,000 *H. contortus*.
Group 2: 3 sheep each receiving 12,750 *T. colubriformis*.
Group 3: 34 sheep each receiving 100,000 *H. contortus* and 12,750 *T. colubriformis*.

It was necessary to slaughter sheep during the prepatent period to serve as indicators of the larval viability and the number of immature worms present. For this purpose the following animals were slaughtered after larval dosage on the days indicated:—

(*a*) One sheep each from Groups 1 and 2 on the second, fourth and tenth days respectively.

(*b*) One sheep from Group 3 on the seventh and another on the fourteenth day.

Of the remaining 32 sheep of Group 3, eight were not treated and served as controls; the balance (24) was divided into six groups of four sheep each. The sheep were allocated to any particular group at random. The sheep in three of these groups were dosed with anthelmintics on the seventh, the other three groups on the fourteenth day after larval challenge.

The anthelmintics were administered at the standard dosage rates recommended i.e. Thiabendazole 50 mg/Kg *per os*, Methyridine 200 mg/Kg subcutaneously, and Coopex 200 mg/Kg *per os*.

The 32 sheep were slaughtered 27 to 30 days after larval infestation, during which time they were maintained under worm-free conditions. At *post mortem* examination each abomasum was collected separately by severing the gut between double ligatures at the pylorus and fundus. Similarly, the first 7 metres of each small intestine were collected separately because careful examination of the slaughtered indicator sheep had shown that *T. colubriformis* was confined to that portion of the alimentary tract.

Results

The results are given in Tables 7 and 8.

TABLE 7.—*Immature worms recovered from slaughtered indicator sheep*

Sheep No.	No. of Larvae dosed		Day slaughtered	Worms recovered Post Mortem	
	H. contortus	*T. colubriformis*		*H. contortus*	*T. colubriformis*
31	100,000	—	2	6,555	—
37	100,000	—	4	17,000	—
34	100,000	—	10	57,150	—
23	—	12,750	2	—	2,900
45	—	12,750	4	—	4,785
53	—	12,750	10	—	5,599
44	100,000	12,750	*7	44,410	4,740
12	100,000	12,750	*14	58,658	8,575

* Experimental sheep drenched on these days.

From Table 7 it can be seen that there was a good " take " of larvae of both *H. contortus* and *T. colubriformis*, whether dosed singly or together, so that it could be anticipated that at the time of dosing the treated sheep would be carrying a worm burden of the order of 50,000 *Haemonchus* and 6,500 *Trichostrongylus*. Reference to Table 8, however, will show that when the control sheep were slaughtered there were present somewhat less than half that number of wire worms. No explanation is offered for this discrepancy nor for the finding that the number of worms recovered with each species progressively increased over the 10 to 14 day period between infestation and slaughter. The latter finding may be due entirely to the technical difficulty of detecting the younger larval forms in the mass of material examined.

From Table 8 it is apparent that Thiabendazole, for all practical purposes was 100 per cent effective against the prepatent stages of both worm species. Methyridine, except in the case of two sheep (No. 50 and 54), was almost equally effective. Coopex on the other hand was slightly less effective than either of the other two drugs particularly against 7-day *Haemonchus* and 14-day *Trichostrongylus*.

TABLE 8.—*Worms recovered from sheep treated 7 to 14 days and slaughtered 27 to 30 days after larval infestation*

Sheep No.	*Haemonchus*	*Trichostrongylus*
GROUP 1 (Controls)—		
14	3,700	9,641
15	23,003	10,769
17	26,203	7,745
18	18,191	7,969
19	23,531	9,285
20	91	12,014
21	17,984	9,669
22	23,693	10,830
Mean	17,049	9,740
GROUP 2a (Thiabendazole)—		
36	8	0
51	4	3
57	0	10
59	2	0
Mean	3	3
% Efficacy	>99·9%	>99·9%
GROUP 2b (Thiabendazole)—		
52	1	0
55	0	0
56	2	3
58	3	0
Mean	1	1
% Efficacy	>99·9%	>99·9%
GROUP 3a (Methyridine)—		
39	8	172
41	46	18
42	8	74
47	0	26
Mean	15	72
% Efficacy	>99·9%	99·3%
GROUP 3b (Methyridine)—		
48	330	15
49	612	5
50	3,299	5
54	895	2,351
Mean	1,284	594
% Efficacy	92·5%	93·9%

Sheep No.	*Haemonchus*	*Trichostrongylus*
GROUP 4a (Coopex)—		
24	1,547	472
27	4,657	83
28	6,200	723
32	32	320
Mean	3,109	399
% Efficacy	81·76%	95·9%
GROUP 4b (Coopex)—		
25	245	1,050
29	964	6,438
33	2	3,193
35	4	752
Mean	304	2,858
% Efficacy	98·2%	70·7%

KEY TO GROUPS.

GROUP 1.—Undosed controls.

GROUP 2.—Dosed with Thiabendazole at 50 mg/Kg *per os*:
(*a*) 7 days after larval challenge. (*b*) 14 days after larval challenge.

GROUP 3.—Injected subcutaneously with Methyridine at 200 mg/Kg:
(*a*) 7 days after larval challenge. (*b*) 14 days after larval challenge.

GROUP 4.—Dosed with Coopex *per os* at 200 mg/Kg:
(*a*) 7 days after larval challenge. (*b*) 14 days after larval challenge.

Mature and immature H. contortus

Materials and methods

For some months three groups, each of 10 Merino weaners, reared and maintained under worm-free conditions, had been dosed with an estimated 2,000, 4,000 and 8,000 larvae respectively per week. Five sheep died and from the number of worms collected *post mortem* it was clear that the number of worms administered per dose was considerably in excess of that estimated. A careful check revealed that a regrettable error had occurred and each sheep had received 2½ to 4 times the estimated dose of larvae. After the last dose of larvae the sheep were divided into three groups:—

(1) Six sheep were dosed with Thiabendazole at 50 mg/Kg *per os*.

(2) Six sheep were treated with Methyridine at 200 mg/Kg intraperitoneally.

Two sheep from each group (1 and 2) were treated 24 hours, the balance 58 hours after the last larval dose.

(3) Thirteen sheep served as undosed controls.

All the sheep were slaughtered from 34 to 58 hours after treatment. After slaughter the contents of the omasum and reticulum, abomasum and duodenum of each sheep were collected separately; the abomasum itself was digested with pepsin. These samples were washed, sieved and examined by the technique described (*vide supra*). Total counts were made on the reticular, omasal and duodenal ingesta

as well as on the digested abomasum. Aliquots of the abomasal ingesta were collected and counted as previously described. If aliquots were negative or contained only a few worms, total counts were made.

The developmental stages of *Haemonchus* were differentiated according to Veglia's (1915) description of the life cycle and classified as third, fourth and fifth stage larvae and adults, although it is believed that a differentiation between fifth stage larvae and adults is of little value in determining anthelmintic efficacy of drugs.

Results

The results are presented in Table 9.

TABLE 9.—*Worms recovered* post-mortem *in a controlled test on immature and mature* H. contortus

Sheep No.	*e.p.g. prior to dosing	Stage of development				Total
		3	4	5	A	
Undosed controls						
5	867	0	6	23	5	34
7	267	53	529	56	22	660
11	133	0	70	1	15	86
12	733	11	363	61	54	489
14	1,000	25	838	202	206	1,271
16	67	20	1,107	80	75	1,282
18	200	5	928	80	15	1,028
23	67	3	556	15	0	574
28	23,467	0	2,414	17,720	4,048	24,182
29	4,333	65	1,509	242	847	2,663
30	400	0	0	0	2	2
32	400	45	260	91	20	416
33	24,200	100	4,876	10,425	6,489	21,890
Mean.....	4,318	25	1,035	2,230·5	907·5	4,198
Thiabendazole 50 mg/Kg *per os*						
4	1,333	0	0	0	0	0
8	133	0	0	0	0	0
13	12,267	0	0	0	0	0
20	467	0	0	0	0	0
25	21,533	0	0	0	1	1
27	9,533	0	0	0	0	0
Mean.....	7,544	0	0	0	0·16	0·16
Methyridine 200 mg/Kg intraperitoneally						
9	2,467	0	0	0	0	0
10	667	0	0	0	0	0
15	733	0	0	0	1	1
19	6,733	0	0	0	1	1
26	800	0	0	0	1	1
31	25,400	0	1	0	5	6
Mean.....	6,133	0	0·16	0	1·3	1·5

* e.p.g. = Eggs per gram of faeces.

It so happened that the mean e.p.g. in the undosed controls was somewhat lower than in the two groups of treated sheep. It is believed that this was quite fortuitous and may be attributable to the larger number of animals in the control group. However, it is considered that any conclusion as to the efficacy of the drug cannot be materially affected. An analysis of the faecal egg counts in individual animals indicates that a single egg count, particularly in animals with less than 1,000 e.p.g., cannot be regarded as a true reflection of the worm burden of the host.

In the control sheep, with three exceptions (Sheep No. 5, 11 and 30), a moderate to heavy infestation of immature worms was shown to be present. The worm burden of adults except in two sheep (No. 28 and 33) was found to be disappointingly low. This finding will be discussed elsewhere but it does not detract from the quite remarkable efficacy of both drugs against both immature and mature *Haemonchus*.

Discussion

The critical test of Hall & Foster (1918) and the controlled test of Moskey & Harwood (1941) form the basis of any slaughter trial for assessing anthelmintic efficacy. These methods and the faecal egg count test were reviewed by Moskey & Harwood (1941) and Gordon (1950). The trials described above attempted to simplify and standardise the critical and controlled test.

The need for short-term critical slaughter trials was suggested by the extremely rapid action of two of the drugs tested; Methyridine caused the expulsion of worms in 16 to 24 hours and Thiabendazole in less than 48 hours.

This method was eminently suited to intestinal parasites which were recovered in large numbers in a well-preserved state distal to their normal habitat. Gordon (1950) stated that the critical test was suited only to caecal and colonic worms. He made no mention of *Trichostrongylus* spp., inhabitants of the abomasum and small intestine. These trials proved that the critical test was very suited to this genus as large numbers of well-preserved worms were found in the caecum, colon and faecal bags. *Trichostrongylus* spp. should therefore be included as a suitable parasite for critical tests.

The unsuitability of short-term slaughter is shown up by slow-acting anthelmintics—or by anthelmintics which are dependent on their cumulative effect. The latter is possibly the case with Coopex, where better results were obtained after 48 hours than at short-term slaughtering. Sheep that are moribund or in an advanced state of parasitism and that do not defaecate are unsuitable experimental animals in that the worms are, even when dead, retained in their normal habitat thus defeating the object of short-term slaughter.

Although the sheep used for trials on *H. contortus* were heavily infested, as shown by the initial high egg counts, very few worms were recovered *post mortem*. The worms were digested on their passage through the small intestine, thus proving the method to be of little value for adults. This finding agrees with the statements of Gordon (1959, personal communication) and Gibson (1961) viz. that critical trials were of little value with *H. contortus*.

The fluctuations and the inconsistency in the results with immature worms can be explained by the fact that these stages are very small, difficult to see and more easily masked by the larger amount of debris in the faeces than by the fine particles of the ingesta. Fourth stage *O. columbianum* may have been killed by the drug(s), but the period till slaughter may have been too short for expulsion from the nodules. Their absence from the faeces may lead to erroneous conclusions as to the efficacy of the drug.

To overcome some of the shortcomings of the trials already discussed two modified controlled tests were carried out, one on immature *H. contortus* and *T. colubriformis*, the other on all parasitic stages of *H. contortus*.

For the immature trials yearlings were infested with two species of infective larvae and some of these sheep drenched when the worms were seven and 14 days old. Since only *H. cortortus* and *T. colubriformis* were involved, only the abomasum and first seven metres of the small intestine needed to be examined *post mortem*. Thirteen to 16 days were allowed to elapse after the last treatment before slaughter, similar to the 14-day period of Moskey & Harwood (1941). The sheep slaughtered during the prepatent period had shown that large numbers of immature worms were still present in all animals at the time of treatment. The scope of the trial was limited to two species only. However, it is considered unlikely that young sheep, heavily infested with more than two species, would survive until the termination of the experiment. Furthermore, it is very doubtful whether all the species would develop equally satisfactorily to adults in a massive mixed infestation. This supposition was clearly illustrated by the severe infestations of *T. colubriformis* and the poor " take " of *H. contortus* in the controls shown in Table 8 when compared with the number of larvae dosed originally.

This trial, however, had grave shortcomings which are obvious if Tables 7 and 8 are compared. The marked discrepancies in the number of worms present during the prepatent period (Table 7), when compared with those recovered from the controls some 13 to 16 days later (Table 8), clearly indicate that the time lag was too long for accurate worm recovery. The controls in Table 8 do not accurately reflect the number of worms present at the time of treatment, as shown by sheep No. 44 and 12 in Table 7. A better method is to slaughter all stock within a few days of treatment.

Banks & Michel (1960) overcame many of the objections to the above procedure by dosing four-months old worm-free calves with 42,000 *Ostertagia ostertagi* larvae each, and then dividing the calves randomly into pairs; one member was treated while the other served as the control. The treated calf was given a single dose of Neguvon either on the seventh, fifteenth, twenty-first, twinty-eighth or twenty-ninth day after larval challenge. Three days after treatment both calves were killed and the worms in their abomasa counted.

These calves were obviously highly susceptible as indicated by the large numbers of worms recovered during the prepatent period. An average of 30,000, with a range of 21,000 to 39,000 worms was recovered, which was very high in relation to the number of larvae dosed. This method recommends itself in that the control calf gives a fair indication of the number of worms present at the time of treatment. An improvement would be large groups of both treated and control calves, e.g. six per group.

The modified controlled test on all stages of development of *H. contortus* (cf. Table 9) had certain unique features which have not been reported previously. The control group consisted of 13 animals and was large enough to overcome any doubts as to the degree of infestation which was generally moderate to severe. For some 10 weeks large larval doses were administered to the sheep every Monday, Wednesday and Friday. Treatment of two animals in each treated group was carried out on Saturday and of four sheep in each group on Sunday evening, i.e. 24 and 58 hours respectively after the last larval dose. This meant that sheep treated on Saturday had immature worms 1, 3, 5, 8, 10, 12, 15 and 17 days old respectively as well as adult worms. If 58 hours can be regarded as approximetely $2\frac{1}{2}$ days then the age of immature worms in the sheep treated on Sunday evening was $2\frac{1}{2}$, $4\frac{1}{2}$, $6\frac{1}{2}$, $9\frac{1}{2}$, $11\frac{1}{2}$, $13\frac{1}{2}$, $16\frac{1}{2}$ and $18\frac{1}{2}$ days respectively, as well as adult worms.

This means that immature stages of all ages were present with the exception of seven and 14 days old worms included in the previous trial and these larval stages covered the whole of the prepatent period not tested in the previous trial. Moreover, the wider distribution was nearer natural conditions than those reported previously (*vide supra*).

Another feature was that the sheep were all slaughtered from Monday to Wednesday following treatment i.e. within 58 hours of treatment. This achieved two objectives. The time lag from treatment to slaughter was less than 2½ days, not 13 to 23 days as in the previous trial. The worms recovered in the controls were therefore a truer reflection of the worms present at the time of treatment. Moreover, sheep were slaughtered from 34 to 58 hours after treatment. In spite of these very short periods the results shown in Table 9 confirm the results on the adult worms in the other trials, viz. the extremely rapid action of Thiabendazole and Methyridine.

The value of anthelmintics effective on all stages of development of parasitic worms need not be stressed. It is essential that parasitologists use reliable methods for assessing the anthelmintic effects on larval as well as adult stages.

Summary and Conclusions

1. The modified critical test, or so-called short-term slaughter test, was the most suitable means of assessing anthelmintic efficacy on adult *Trichostrongylus* spp. and *O. columbianum*. It has some value for *O. circumcincta* but is unsatisfactory on *H. contortus*.

2. The modified controlled test was the best method of testing the effects of anthelmintics on immature worms. It is the method of choice on all stages of development of *H. contortus*.

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