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OVERBERG RESEARCH PROJECTS. XI. FIRST STAGE LARVAL REDUCTION TEST TO ASSESS ANTHELMINTIC EFFICACY ANTE MORTEM IN SHEEP

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ABSTRACT

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Two field trials, one with suckling Merino ewe lambs and the other with yearling Dohne Merino rams, are described. In these the anthelmintic efficacy of febantel (a benzimidazole), ivermectin, levamisole and morantel are compared, using the 1st stage larval reduction test.

The mean natural log (+1 for zero values) of the post treatment larval counts of the treated groups was compared with that of the untreated controls and the percentage reduction used to assess anthelmintic efficacy.

Febantel was only 87,4 % effective against *Teladorsagia* in suckling lambs but the other anthelmintics were more than 99 % effective against this genus. Efficacy against *Haemonchus* and *Trichostrongylus* ranged from 93,2 %–100 % for all 4 compounds. In the rams all compounds were 100 % effective against *Trichostrongylus*, with the exception of morantel which was only 87,5 % effective. None of the compounds were effective against *Teladorsagia*, particularly morantel, animals treated with which having more larvae than the controls.

The interpretation of anthelmintic efficacy; the advantages of the first stage larval reduction test, compared with the faecal egg count reduction test; and the importance of incubating cultures at 30 °C for 24 h, in order to harvest first stage larvae, are discussed.

INTRODUCTION

Differential faecal worm egg counts formed an integral part of the techniques used in studies on the epidemiology of nematode parasites of suckling lambs, hoggets and ewes in the winter rainfall region of the southern Cape (Reinecke & Louw, 1989; Louw, 1989). Numerous cultures (n=471) were made from rectal faeces collected at necropsy from ewes, ram lambs and wethers which were slaughtered for epidemiological studies, in an attempt to harvest larvae for generic identification. Whitlock's (1956) inner-tube method for harvesting infective larvae was applied, or cultures were prepared in glass jars, either mixed with or without vermiculite (Reinecke, 1983) and the incubator temperatures adjusted to 20, 26 or 30 °C.

All these methods proved to be unsatisfactory as the number of larvae harvested was often low, even when high worm egg counts had been recorded, and it was impossible to correlate the genera and number of infective larvae harvested by these means with the species and number of worms recovered from the same sheep post mortem. Of 151 cultures made from faeces collected every 6 weeks from the same ewes and ewe lambs, only 85 were positive for *Teladorsagia*, despite the presence of this genus in 47 out of 48 wether lambs and hoggets slaughtered in groups of 6 per group every 6 weeks. The latter grazed with the ewes throughout the period when ewes were sampled for faecal egg counts (Reinecke & Louw, 1989). It was therefore necessary to seek another method of diagnosing nematode parasites in live sheep.

Reinecke (1990) described a method of identifying first stage larvae per gram (L_1p.g.) of faeces. The dominant genera in the winter rainfall area are *Teladorsagia*, *Trichostrongylus* and *Nematodirus*, which have a low fecundity, and in order to obtan sufficient larvae, it was necessary to standardise the faecal mass examined at 5 g for hoggets and adult sheep and express the results as L_1p.5 g. In suckling lambs, eggs were more concentrated and frequently only a few pellets (or a small quantity of slimy faeces) could be collected, in which case 1st stage larvae per gram (L_1p.g.) were estimated, rather than L_1p.5 g. used in older sheep, for reasons given above.

The first stage larval reduction test (L_1RT), used to monitor worm resistance to anthelmintics, is described in this paper. Two field trials, one on suckling lambs and the other on rams, are described in some detail as examples of the techniques used for the different age groups of sheep. The importance of incubating cultures long enough to allow L_1 to hatch is stressed and data are presented to show that cultures must be incubated for 24 h at 30 °C for more than 75 % of L_1 to hatch. The advantages of the L_1RT, compared with the faecal egg count reduction test, are discussed.

1. L_1RT in lambs

MATERIALS AND METHODS

Procedures for collecting faeces in the field, randomizing sheep, etc., described by Presidente (1985) and Anderson (1989) for the FECRT, were followed.

Farm, animals and grazing

On the farm Kliprivier, Swellendam, 100 suckling Merino ewe lambs were herded into kraals on 24 July 1990 and the mass of 20 of the largest lambs determined. The lamb with the greatest mass had a body mass of 36 kg and the oral dose of anthelmintics was based on this mass, as follows:

Febantel (FBL) 5 mg kg^{-1}	Rintal (Bayer) 7 mℓ
Ivermectin (IVM) 0,2 mg kg^{-1}	Ivomec (Logos) 9 mℓ
Levamizole (LVZ) 7,5 mg kg^{-1}	Ripercol I (Janssen) 9 mℓ
Morantel (MRL) 12,5 mg kg^{-1}	Banminth II (Pfizer) 9 mℓ

Lambs were randomized and 18 were allocated to each of the 4 treated groups as well as to the untreated control group.

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TABLE 1 Merino ewes and lambs at Kliprivier, Swellendam. Anthelmintics dosed and history of ewes and lambs prior to L_1p.g. trial

1989

1 November – 4 December: Ewes served by rams.

1990

Ewes: dosed with levamisole LVZ Ripercol *1* (Janssen) once; albendazole ABZ Quadrazole (Janssen) once; morantel MRL Banminth II (Pfizer) once; clostridial toxoids + levamisole Ovivax L Hidose (Coopers) + Se injection 3 ×.
Lambing started 1 April.

Lambs: ewe lambs dosed with nicosamide NCL Lintex (Bayer) once: ram lambs dosed with rafoxanide RFX Ranide (Logos) once.
Grazed in lucerne, clovers, barley (55 ha) or oats (124 ha) or dry-land lucerne (264 ha).

The nose or poll of each animal was marked with raddle of different colours for each group; faeces were collected from the rectum of each lamb and placed separately in labelled 60 mℓ plastic collection jars (Becker, Johannesburg). The faecal specimen jars were surrounded by ice cubes packed in a polystyrene container (Hebcooler) and transported to the laboratory. The mass of each specimen was determined and recorded (Table 2) and each faecal specimen prepared for the recovery of eggs and L_1, as described by Reinecke (1990).

The cultures were incubated at 30 °C for 24 h by which time more than 75 % of the eggs had hatched. The fluid from the various flat sided culture bottles was poured into separate 25 mℓ labelled bottles to which concentrated I_2 solution was added to kill the larvae plus a few drops of formalin to fix them. These bottles were placed on a bench for at least 10 min to allow the larvae to settle, the supernatent discarded leaving about 5 mℓ of liquid in each bottle, and the sediment in each bottle examined for L_1 with the aid of a stereoscopic microscope. If there were less than 50 L_1 present, the fluid was poured into a smaller polytop bottle (7 mℓ) which was then placed inside the labelled larger bottle, the larvae allowed to settle (>10 min), the supernatent discarded, leaving ± 1 mℓ of fluid which was pipetted into the counting chamber(s). The number of eggs and L_1 in the entire specimen were counted under a compound microscope using a white blood cell counter with 6 keys (Clay Adams).

Since the larvae in 3 chambers of the counting slide were counted and L_1 and eggs on the grids of each chamber represented the number present in 0,1 mℓ of the suspension, the fluid in the polytop bottle was adjusted to multiples of 3 mℓ to simplify counting. Thus, after a rough estimate of L_1 present in the polytop bottle, water was added as follows:

L_1 estimated	Volume	Total
50–≤ 500	3 mℓ	× 10
500–1000	6 mℓ	× 20
> 1 000	9 mℓ	× 30

The number of L_1 counted was converted to L_1p.g. as described by Reinecke (1990).

Interpretation of the results

The mean natural log (+1 for zero values) of the post treatment larval counts of the treated groups was compared with that of the untreated controls and the percentage reduction for Group 2–5 listed in Table 2. In addition to the geometric means (G) the arithmetic means and standard deviations are included in this table.

Anthelmintics dosed to ewes and lambs

The history of the flock prior to the L_1RT including the anthelmintics dosed to both ewes and lambs is summarized in Table 1.

RESULTS

Controls (Group 1, Table 2)

On 31 July all the lambs had *Teladorsagia* (Range 160–4850 L_1p.g.), *Haemonchus* was present in 16/17 (range 10–3150 L_1p.g.) with lower counts of *Trichostrongylus* (range 6–150 L_1p.g.) but only 2 of 17 sheep were infected with *Oesophagostomum*.

Anthelmintic efficacy (Table 2)

Sixteen out of 17 lambs dosed with FBL had *Teladorsagia,* the percentage reduction was only 87,4 % and this compound was not effective. The efficacy against *Haemonchus* ranged from 97 % (IVM) to 100 % (LVZ) if interpretation is based entirely on percentage reduction of the geometric means. It must be emphasized that this interpretation is probably misleading because all the lambs, 18/18 treated with IVM had 1–70 L_1p.g. of *Haemonchus* (Group 3, Table 2). This will be discussed below.

2. L_1RT in adult sheep

MATERIALS AND METHODS

Farm, animals and grazing

Uitkyk (Caledon) has a stud of Dohne Merinos which grazed on spray-irrigation Kikuyu/legume pastures. On 27 November 1990, the day before the trial, the mass of 70 yearling rams was determined. The ram with the greatest mass had a body mass of 60 kg and based on this mass, 60 rams were randomized and dosed as follows:

Group 6:	Undosed controls		
Group 7:	Rintal FBL	12 mℓ	12 rams
Group 8:	Ivomec IVM	15 mℓ	12 rams
Group 9:	Ripercol *1* LVZ	15 mℓ	12 rams
Group 10:	Banminth II MRL	15 mℓ	12 rams

Faecal samples were collected, transported to the laboratory and 5 g specimens mass-measured and processed as described above. After a rough estimate of the number of L_1 present in the polytop bottle, water was added as follows:

L_1 estimated	*Volume*	*Total*
50–100	3,75 mℓ	× 12,5
100–< 500	7,5 mℓ	× 25
500–1 000	15 mℓ	× 50
> 1 000	30 mℓ	× 100

Since L_1 and eggs were counted in 3 chambers × 0,1 = 0,3 mℓ and 5 g of faeces were collected, the factors by which the total L_1 of each genus + eggs was multiplied = L_1 and eggs per 5 g. If there were < 50 L_1 the same procedure described earlier was followed.

RESULTS

Controls (Table 3)

Low L_1p.5 g. counts were recorded for *Teladorsagia* and *Trichostrongylus* and 11/12 and 9–12 rams were infected respectively on 10 December. Al-

TABLE 2 L_ip.g. in 5 groups of lambs at Kliprivier

	Egg	eme	N	H.c.	Te	Tr	Ov	Total	Mass
24 July 1990									
Group 1: Undosed controls									
n	16	4	4	12	18	15	4	18	18
r	1–	6–	5–	1–	1–	1–	1–	1–	1,6–
	136	43	13	388	562	360	30	1 150	5
x	37	4	2	58	178	45	3	327	–
sd	44	11	4	94	161	88	8	313	–
31 July 1990									
n	16	9	6	16	17	17	2	17	17
r	12–	10–	5–	10–	137–	6–	20–	189–	0,7–
	2 150	150	60	3 150	4 850	150	60	10 450	5
x	242	23	8	329	769	74	5	1 450	–
sd	522	42	16	738	1 005	40	5	2 388	–
G	67	5	3	99	493	59	1,5	854	–
24 July 1990									
Group 2: Dosed with febantel FBL Rintal (Bayer) at 5 mg kg^{-1} *per os*									
n	16	2	12	14	18	15	3	18	18
r	1–	1–	5–	3–	6–	3–	6–	9–	1,4–
	200	5	22	154	517	77	20	803	5
x	35	0,3	7	40	213	28	1,8	326	–
sd	53	1,2	7	48	156	25	5	269	–
31 July 1990									
n	16	8	8	6	16	9	1	16	17
r	1–	1–	1–	2–	1–	1–	9	5–	0,3–
	178	83	30	89	685	116	–	979	5
x	41	9	5	8	176	13	–	252	–
sd	56	20	8	21	199	28	–	295	–
G	15	3	2	2	62	4	0	102	–
red	77,6	40	33,3	98	87,4	93,2	–	88,1	–
24 July 1990									
Group 3: Each lamb dosed with invermectin IVM Ivomec (Logos) at 0.2 mg kg^{-1}									
n	12	2	9	18	18	17	6	18	18
r	1–	5–	8–	5–	22–	1–	1–	44–	1,5–
	200	10	30	460	763	110	17	1 301	5
x	30	0,8	7	84	249	30	2	403	–
sd	49	3	8	116	190	31	4	398	–
31 July 1990									
n	15	3	1	18	12	3	0	18	18
r	1–	1–	1	1–	1–	1–		2–	0,8–
	95	5	–	70	14	1	–	170	5
x	13	0,5	–	16	2	0,2	0	32	–
sd	25	1,3	–	19	4	0,4	–	44	–
G	4	0,1	0	3	1,8	0	0	14	–
red	94	97	100	97	99,6	100	100	98,4	–
24 July 1990									
Group 4: Each lamb dosed with levamisole LVZ (Ripercol *l* at 7,5 mg kg^{-1} *per os*									
n	13	2	7	15	17	15	8	17	17
r	1–	5–	5–	1–	12–	2–	5–	15–	1,0–
	50	6	38	112	525	89	38	738	5
x	14	0,6	5	37	178	27	8	270	–
sd	13	2	10	40	156	29	12	228	–
31 July 1990									
n	7	2	2	4	14	2	0	15	16
r	1–	1–	1–	1–	1–	20–	–	1–	0,7–
	6	1	1	1	210	20	–	235	5
x	1	0,1	0,1	0,2	20	2,3	–	24	–
sd	2	–	–	0,4	52	–	–	59	–
G	1	0	0	0	3,3	1,4	0	4	–
red	98,5	100	100	100	99,3	97,4	100	99,5	–
24 July 1990									
Group 5: Each lamb dosed with morantel MRL (Pfizer) at 12,5 mg kg^{-1} *per os*									
n	13	4	5	13	18	17	3	18	18
r	5–	10–	1–	10–	1–	1–	1–	1–	1,9–
	230	20	25	650	680	200	13	1 670	5
x	48	3	3	112	228	43	1	438	–
sd	71	6	7	197	241	60	4	533	–
31 July 1990									
n	5	2	2	6	16	2	0	16	17
r	1–	1–	1–	1–	1–	1–	–	1–	1,8–
	1	1	1	3	110	5	–	115	5
x	0,3	–	0,1	–	12	0,3	0	13	–
sd	0,5	–	–	0,9	26	1,2	–	27	–
G	0	0	0	1	4	0,1	0	5	–
red	100	100	100	98,9	99,2	99,8	100	99,4	–

* Table headings: egg = unhatched; eme = embryonated eggs; N = *Nematodirus* eggs. H.c. = *Haemonchus*; Te = *Teladorsagia*; Tr = *Trichostrongylus*; Ov = *Oesophagostomum* first stage larvae; Total = eggs + L_i; mass g = number of faecal samples examined + range of the mass of faeces in grams. First column: n = number positive; r = range of positive results; x = arithmetic mean; sd = standard deviation; G = geometric mean; red. = percentage reduction when the G of the treated group is compared with that of the G in the undosed controls

TABLE 3 L_1p.5 g. in 5 groups of rams at Uitkyk*

	Egg	eme	N	H.c.	Te	Tr	Ov	Total	No. of samples
28 November 1990									
Group 6: Undosed controls									
n	5	8	4	6	11	4	0	11	12
r	1–	1–	1–	1–	1–	1–	–	4–	–
	12	68	1	50	175	25	–	250	–
x	2	12	0,3	5	48	2	0	69	–
sd	3	20	0,5	14	58	7	–	82	–
10 December 1990									
n	2	1	1	2	11	9	9	12	12
r[1]	12–	1	25	1–	6–	1–	1–	7–	–
[2]	50	–	–	2	275	150	75	375	–
x	6	–	–	–	69	27	14	116	–
sd	14	–	–	–	78	41	20	112	–
G	1	–	–	–	29	8	6	62	–
28 November 1990									
Group 7: Each ram dosed FBL at 5 mg kg^{-1} *per os*									
n	6	8	3	4	10	3	0	11	12
r	1–	1–	1–	13–	1–	1–	0	2–	–
[3]	125	75	25	112	1 300	30	0	1 600	–
x	14	22	2	22	277	2	0	339	–
sd	34	28	7	39	390	390	0	502	–
10 December 1990									
n	2	3	0	3	11		0	11	12
r	1–	1–	–	1–	1–	–	–	1–	–
	50	25	–	25	425		–	500	–
x	4	3	0	2	48	–	–	57	–
sd	14	7	–	7	117	–	–	138	–
G	–	–	–	–	6	0	0	7	–
red	–	–	–	–	79	100	100	88,7	–
28 November 1990									
Group 8: Each ram dosed IVM at 0,2 mg kg^{-} *per os*									
n	6	7	1	5	12	8	0	12	12
r	1–	2–	25–	4–	1–	1–	–	1–	–
[4]	25	400	–	25	700	175	–	925	–
x	13	55	2	7	148	22	–	247	–
sd	21	112	–	11	193	47	–	290	–
10 December 1990									
n	1	1	1	1	4	0	0	4	12
r	*13	*100	2	*62	1–	–	–	1–	–
[5]	–	–	–	–	†425	–	–	†600	–
x	1	9	0	5	36	–	0	51	–
sd	–	–	–	–	122	–	–	173	–
G	–	–	–	–	1,9	0	0	2	–
red	–	–	–	–	93,4	100	100	96,8	–
28 November 1990									
Group 9: Each ram dosed LVZ at 7,5 mg kg^{-1} *per os*									
n	4	7	2	7	9	4	0	10	11
r	3–	1–	1–	1–	8–	3–	–	8–	–
[6]	25	200	25	300	2 700	200	–	3 200	–
x	4	28	2	47	344	22	0	447	–
sd	8	59	7	93	799	59	–	948	–
10 December 1990									
n	2	0	0	3	10	0	0	11	11
r	1–	–	–	1–	2–	–	–	2–	–
	13	–	–	8	138	–	–	138	–
x	1	–	–	1	43	0	0	45	–
sd	–	–	–	–	51	–	–	51	–
G	–	–	–	–	15	0	0	17	–
red	–	–	–		48,3	100	100	72,6	–
28 November 1990									
Group 10: Each ram dosed MRL at 12,5 mg kg^{-1} *per os*									
n	5	7	0	5	10	4	0	11	11
r	1–	1–	–	4–	1–	2–	–	1–	–
	25	100	–	50	700	100	–	850	–
x	4	28	–	41	260	19	–	352	–
sd	9	42	–	109	333	37	–	556	–
10 December 1990									
n	1	4	0	3	10	1	0	10	11
r	75	25–	–	3–	5–	50	–	3–	–
	–	375	–	25	900	–	–	1 000	–
x	–	48	–	5	247	–	0	311	–
sd	–	111	–	10	270		–	298	–
G	–	–	–	–	70	1	0	92	–
red	–	–	–	–	+141,4	87,5	100	+48,4	–

*Abbreviations: See Table 2. Faecal samples = 5 g per sample

[1] Sheep 215 had 25 *Strongyloides* [2] Sheep 221 had 1 *Strongyloides* [3] Sheep 419 had 12 *Strongyloides* [4] Sheep 410 had 25 *Strongyloides* [5] Sheep 11 had 25 *Strongyloides* † = Sheep 11. These maximum egg, L_1 and total counts indicate that this sheep was probably not drenched (see text). [6] Sheep 408 had 1 *Strongyloides*

though *Oesophagostomum* was absent on the treatment day in November 8/11 rams were infected with this genus on 10 December. This was probably due to L_4 and 5th stage females of *Oesophagostomum* developing to adults in the 12 days that had elapsed between 28 November and 10 December. Two sheep in the controls had *Strongyloides* on 10 December (Table 8).

Anthelmintic efficacy (Table 3)

All compounds were 100 % effective against *Oesophagostomum* and FBL, IVM and LVZ similarly effective against *Trichostrongylus*. In Group 10, only Ram 147 (which was treated with MRL) had *Trichostrongylus*. Three anthelmintics were not effective against *Teladorsagia,* the G mean reduction for FBL being 79,0 % (9/12 infected) and for LVZ 48 % (9/11 infected). Only 4/12 rams dosed with IVM in Group 8 had *Teladorsagia*. Ram 11 had 425 L_1 of *Teladorsagia* which accounted for the reduction of 93,1 % (G mean) and 47,8 % (arithmetic mean) respectively. This should be ignored, only 3/11 of the other rams in Group 8 having 1, 2 and 3 L_1 of *Teladorsagia*. Rams in Group 10 treated with MRL had more larvae than most of the controls and an increase in mean larval counts of +258,0 % (G mean) and +141,4 % (G mean) respectively. A few *Strongyloides* L_1 were recovered from 1 or 2 sheep in all the other groups (except Group 10) sampled on 28 November.

This flock was overdosed, being drenched every month (excluding June) from 20 October 1989–18 October 1990. The drenches in descending order were MRL 5 ×, IVM 2 ×, LVZ 2 ×, Closantel CSL 2× and rafoxanide RFX + Thiabendazole + TBZ 1 ×. Dosing MRL frequently probably accounted for the inefficacy of this drug against *Teladorsagia*.

Discussion

Martin (1988) stated, "For unequivocal interpretation of the results, pre-treatment and control geometric mean egg counts should exceed 100 eggs per gram (e.p.g.) and there should not be any zero counts pre-treatment".

In the present trials only the control lambs had G mean counts of 493 L_1p.g. for *Teladorsagia*, falling to 99 and 59 L_1p.5 g. for *Haemonchus* and *Trichostrongylus* respectively (Group 1, Table 2). The rams only had a G mean count of 29 L_1p.g. for *Teladorsagia* in the controls (Group 6, Table 3) but this rose to 70 L_1p.5 g. in the group treated with MRL (Group 10, Table 3). Despite increasing the faecal mass to 5 g for the rams, these results still do not fulfill Martin's (1988) requirements.

Females of the dominant genera in the winter rainfall area, *Teladorsagia* and *Trichostrongylus* lay very few eggs. Reinecke & Groeneveld (1991) compared worm egg counts with nematode worm burdens from more than 400 sheep killed in experiments on the epidemiology of these parasites in this region. The G mean egg counts gave a rough prediction of the G mean worm counts in groups of sheep, and this was the best method of predicting worm counts. Presidente (1985) stated that both *Trichostrongylus* and *Teladorsagia* did not have a good correlation between faecal egg counts and worm counts, which was confirmed by Reinecke & Groeneveld (1991).

Field trials to test anthelmintics by the faecal egg count reduction test (FECRT) (Presidente, 1985; Anderson, 1989) are widely used in Australia. In 1989 I did 4 field trials in the Cape, using FECRT, and also killed 3 sheep per group for controlled anthelmintic tests (CAT) to compare the egg counts with worms recovered at necropsy (Reinecke, unpublished observations, 1989). These, combined with FECRT and CAT tests merely confirmed the findings of Presidente (1985) and Reinecke & Groeneveld (1991) that faecal egg counts were not an accurate method of estimating worm burdens in sheep. Many sheep with G mean egg counts of <100 have worm counts of >1 000 adult *Teladorsagia* and *Trichostrongylus* and since these are the dominant genera, G mean egg counts exceeding 100 which Martin (1988) has specified, are not realistic in the field.

The advantages of L_1RT, compared with the faecal egg count reduction test FECRT, are as follows:

(1) All genera present will yield L_1, even those where only a few adult females are present, e.g. *Oesophagostomum* and *Strongyloides* (Tables 2 and 3) which is not the case where infective larvae are harvested from combined cultures of 10 sheep per group in the FECRT.

(2) Nematode genera can be identified after 24 h incubation at 30 °C. It is not necessary to first do an egg count, harvest infective larvae from cultures 7–10 days later, identify the larvae and convert the egg count to the genera present, as is the case with differential faecal egg counts used in the FECRT.

(3) A single examination of L_1 obviates incorrect conclusions if a sheep fails to swallow the anthelmintic or it is not dosed, as was the case with Sheep 11 which had 62 and 425 L_1 of *Haemonchus* and *Teladorsagia* respectively when sampled on 10 December 1990 (Table 10). Because of these L_1 counts the percentage reduction in the G mean of *Teladorsagia* was only 93,1 % when compared with the controls. In the FECRT recommended by Anderson (1989), the faeces of the entire group is pooled and this may lead to a false interpretation of efficacy, particularly as egg counts are standardized on an e.p.g. basis and *Teladorsagia* is a poor egg layer.

(4) Except in lambs where L_1 are more concentrated, the use of L_1p.5 g. of faeces in weaned lambs and older sheep means that L_1 of *Teladorsagia* and *Trichostrongylus* are more likely to be recovered than if all larval counts were standarised at L_1p.g. This may explain why L_1 counts are more efficient than faecal egg counts standardized on an e.p.g. basis.

(5) Because little or no faeces are present, eggs and L_1 are easily visible microscopically. It is necessary to use 4 g of faeces in 30 mℓ of sugar solution to estimate low egg counts (17 e.p.g.) for the FECRT. The microscopic fields are darker, eggs are either difficult to see or not seen at all microscopically, which inevitably leads to false egg counts in the FECRT.

Interpretation of results

In a treated group a few L_1 of a particular genus may consistently be present in all sheep and either absent or only present in a few sheep in other treated groups. A good example is *Haemonchus* at Kliprivier, in specimens collected on 31 July, 7 days after treatment. All 18 sheep treated with IVM had *Hae-*

monchus (Group 3, Table 2) whereas the numbers infected were only 6/17 of those treated with FBL (Group 2, Table 2), 4/16 with LVZ (Group 4, Table 2) and 6/17 with MRL (Group 5, Table 6) respectively. Despite a 95 % reduction of *Haemonchus* in the G mean of Group 3 (Table 2) treated with IVM, when compared with the undosed controls Group 1 (Table 2), the recovery of L_1 from all the treated sheep probably indicated that *Haemonchus* was resistant to IVM.

Other methods of analysis of these results may be more suitable when a genus is consistently present in a group of treated sheep. Groeneveld (pers. comm. 1970, cited by Reinecke, 1983) used the binomial method to analyse the results for anthelmintic tests on cestodes, in which more emphasis is placed on the frequency with which all tapeworms are expelled in sheep that are treated. If there is still any doubt as to the efficacy of a compound against nematode parasites, a controlled anthelmintic test or the non-parametric method should be used to confirm the efficacy at necropsy (Reinecke, 1983).

Egg hatching

The time taken to process 70–80 specimens in the laboratory was 3–4 h before all the cultures, in flat-sided medicine bottles, could be placed in the incubator. After 2 h the cultures were placed on the shelves for incubation, from top to bottom, and the temperature was adjusted to 30 °C. After 22 h incubation, 10 cultures were taken from the top shelf, a drop from each was checked and because 97,6 % of the eggs had hatched, it was assumed that all eggs had hatched in all cultures. The eggs were therefore removed, killed with I_2 and fixed with formalin. However, because the thermostat was situated at the top, there was a temperature variation within the incubator. It transpired that only 51,6 % of eggs had hatched in cultures which had been placed at the bottom of the incubator because they had only been incubated for 19 h at 28 °C.

Subsequently, cultures were incubated for 22–24 h at 30 °C but *8–10 cultures were removed from the bottom, rather than the top of the incubator,* a drop from each culture being examined micoscopically. Only if > 75 % of the eggs had hatched could all cultures be removed from the incubator for processing, as described by Reinecke (1990).

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