How Repeatable is a FECRT?

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Introduction

The recently completed national survey on the prevalence of anthelmintic resistance in sheep and beef cattle utilised the faecal egg count reduction test (FECRT) to measure the efficacy of different anthelmintic treatments. An efficacy of less than 95% reduction in faecal nematode egg count (FEC) was taken as being the threshold for defining resistance (McKenna 1994). Thus an efficacy of 94% was taken as indicative of resistance being present while an efficacy of 96% was not.

In a survey involving a large number of farms, variability of the test procedure is unlikely to dramatically change the outcome in terms of measured prevalence, given that farms which are incorrectly classed as falling below the 95% efficacy threshold are likely to be balanced by those which are incorrectly classed as falling above the same threshold. However, for an individual farm(er) the consequences of being incorrectly classified as either resistant or susceptible can be more important. The purpose of this study, therefore, was to address the question of "How repeatable is a FECRT?" The intention was to supply veterinarians and other advisors, likely to be involved in conducting FECRTs, with sufficient information to apply some level of confidence around a diagnosis of resistance following a FECRT.

Methodology

Two commercial farms were selected on the basis of having previously failed a FECRT; the first with ivermectin and the second with albendazole. On each farm at least 250 lambs, running together as a single mob, were set aside for the trial. Routine monitor samples from 15 randomly selected animals were obtained from the flock to determine FEC. Once the mean of these exceeded 350 eggs per gram (epg) and there were no zero counts the trial commenced.

On the first visit at least 250 animals were individually ear-tagged and sampled for FEC. These counts were used to sort the animals into 24 groups of 10 in 3 stages. First the animals were split into 3 groups of 80 based on their FEC; those with high, medium and low egg counts. Second, within each of these levels the 80 animals were further divided into 4 replicate mobs of 20 (each with the same mean count) and third, each of these replicates was further split into 2 groups of 10; those that would be drenched and those that would remain as untreated controls (Table 1).

On the second visit (the pre visit) all animals were faecal sampled and those in the drenched groups were dosed to their individual live weight with the appropriate anthelmintic; Farm 1, ivermectin at 0.2 mg/kg (Ivomec liquid for sheep & goats, Merial NZ Ltd) and Farm 2, albendazole at 4.75 mg/kg (Albendazole, Ancare NZ Ltd). Ten days after treatment (McKenna 1990) all animals were re-sampled for FEC and all were drenched with an effective anthelmintic.

Faecal egg counts were conducted using a modified McMaster method in which 1 egg counted represents 50 epg. The remaining faeces from each group of 10 animals were pooled and cultured for at least 14 days at 20°C – Cultures were undertaken for all 24 groups from both pre- and post-drenching samples. Larvae were extracted by baermannisation and the first 100 from each culture were identified to genus and counted (*Nematodirus* larvae were not included in the 100).

Table 1. Trial design showing how the 240 lambs were divided into 24 mobs of 10.

Level	**1**	**2**	**3**	**4**
High FEC	10 lambs drenched	10 lambs drenched	10 lambs drenched	10 lambs drenched
	10 lambs undrenched	10 lambs undrenched	10 lambs undrenched	10 lambs undrenched
Medium FEC	10 lambs drenched	10 lambs drenched	10 lambs drenched	10 lambs drenched
	10 lambs undrenched	10 lambs undrenched	10 lambs undrenched	10 lambs undrenched
Low FEC	10 lambs drenched	10 lambs drenched	10 lambs drenched	10 lambs drenched
	10 lambs undrenched	10 lambs undrenched	10 lambs undrenched	10 lambs undrenched

Efficacy of the undifferentiated FECRT was calculated using the three equations (listed below) to look at the variation not only within the flock but also the variation between equations (Presidente 1985). Arithmetic means were used in all cases (Dash et al 1988; McKenna 1990).

Equation 1.

$$\left(1-\left(\frac{posttestFEC}{pretestFEC}\right)*\left(\frac{precontrolFEC}{postcontrolFEC}\right)\right)*100$$

Equation 2.

$$\left(1-\left(\frac{posttestFEC}{pretestFEC}\right)\right)*100$$

Equation 3.

$$\left(1-\left(\frac{posttestFEC}{postcontrolFEC}\right)\right)*100$$

Efficacy was also calculated against each genus using equation 4. This uses the culture results to allocate pre- and post-treatment FECs to each genus in both the treated and control groups before applying the Presidente equation (equation 1) for calculating efficacy.

For calculating efficacies, only cultures from the post-treatment visit (treated and control) were used on the assumption that when a control group is present a pre-treatment culture is not usually done. For each of the 12 tests the mean pre-treatment FEC (of the treated group) was

multiplied by the proportion of each genus in the post-treatment control culture to give an estimate of the epg allocated to that genus. Similarly the post-treatment FEC of the treated group was multiplied by the proportion of each genus in the post-treatment treated culture to give an estimated of the epg allocated to each genus after treatment. These were then adjusted for any change in FEC in the control group to give an efficacy against each genus (Equation 4).

Equation 4.

$$\left(1-\left(\frac{\left(posttestFEC\times\left(\%posttestculture/100\right)\right)}{\left(pretestFEC\times\left(\%controlculture/100\right)\right)}\right)*\left(\frac{precontrolFEC}{postcontrolFEC}\right)\right)*100$$

Cultures from the first (pre-treatment) visit were used to evaluate the variability in faecal culture results.

Results

Efficacies determined by overall FEC reduction using equations 1-3 above are presented in Tables 2 and 3, for farms 1 and 2 respectively. The average pre-treatment FEC for each level on farm 1 was; low = 243 epg, medium = 406 epg and high = 620 epg, and on farm 2 was; low = 199 epg, medium = 580 epg and high = 1123 epg.

Table 2. Efficacies of the undifferentiated FECRT calculated using equations 1-3 for farm 1.

		% efficacy		
	Test no.	Equation 1	Equation 2	Equation 3
Low	1	94	76	93
Low	2	92	74	93
Low	3	91	57	94
Low	4	87	52	89
Medium	5	88	66	85
Medium	6	96	85	96
Medium	7	96	81	95
Medium	8	92	75	92
High	9	91	76	87
High	10	84	71	85
High	11	94	74	93
High	12	96	90	95

Table 3. Efficacies of the undifferentiated FECRT calculated using equations 1-3 for farm 2.

		% efficacy		
FEC level	Test no.	Equation 1	Equation 2	Equation 3
Low	1	99	95	99
Low	2	99	95	99
Low	3	95	92	96
Low	4	92	84	96
Medium	5	92	89	96
Medium	6	98	98	98
Medium	7	97	89	97
Medium	8	95	92	97
High	9	96	95	96
High	10	99	98	99
High	11	98	95	97
High	12	98	97	98

The proportions of each parasite genus present in the 24 faecal cultures conducted pre-treatment are presented in Figures 1 and 2.

Figure 1. The proportion of different parasite genera in bulked faecal cultures (10 lambs per culture) collected pre-treatment on Farm 1. *Nematodirus* larvae were excluded, Haem = *Haemonchus*, LT's = *Oesophagostomum* / *Chabertia*, Trichs = *Trichostrongylus*.

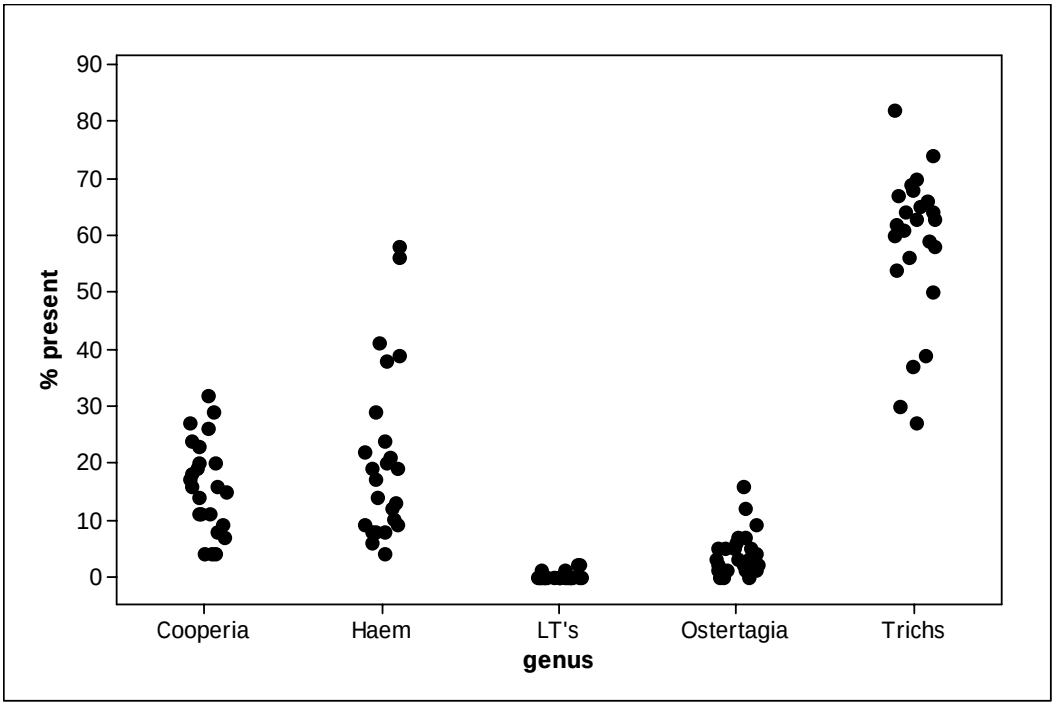
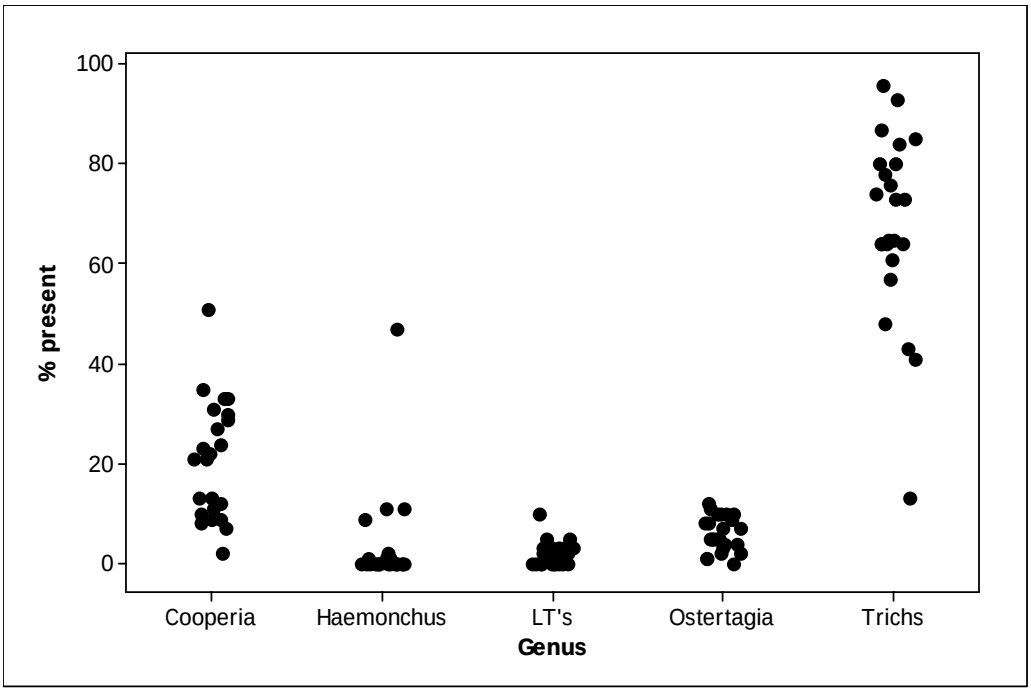


Figure 2. The proportion of different parasite genera in bulked faecal cultures (10 lambs per culture) collected pre-treatment on Farm 2. Nematodirus larvae were excluded, Haem = *Haemonchus*, LT's = *Oesophagostomum* / *Chabertia*, Trichs = *Trichostrongylus*.



Tables 4 and 5 give the estimated efficacies against the main genera present using the culture results. In addition, the estimated FEC for each genus present in the test is also given - this is calculated by multiplying the mean pre-treatment FEC (of the treated and control groups for each test) by the proportion of each genus present in the post-treatment control cultures.

Table 4. Farm 1 - FEC and efficacies apportioned to each genus on the basis of culture results from that test. Efficacies are calculated using equation 4

FEC Level	Test no.	*Cooperia* FEC	Efficacy against *Cooperia*	*Ostertagia* FEC	Efficacy against *Ostertagia*	*Trichostrongylus* FEC	Efficacy against *Trichostrongylus*
Low	1	43	74	13	65	156	100
Low	2	39	69	21	76	157	99
Low	3	37	76	9	0	108	100
Low	4	22	17	16	51	111	99
Medium	5	23	81	18	0	234	97
Medium	6	42	83	5	13	232	98
Medium	7	55	86	18	72	197	99
Medium	8	61	79	22	31	162	95
High	9	88	85	26	0	344	96
High	10	13	0	19	0	338	99
High	11	97	87	18	29	231	98
High	12	49	80	40	59	583	100

Table 5. Farm 2 - FEC and efficacies apportioned to each genus on the basis of culture results from that test. Efficacies are calculated using equation 4

FEC Level	Test no.	Cooperia FEC	Efficacy against Cooperia	Ostertagia FEC	Efficacy against Ostertagia	Trichostrongylus FEC	Efficacy against Trichostrongylus
Low	1	76	99	11	100	62	100
Low	2	46	98	10	100	184	100
Low	3	54	92	0		129	100
Low	4	75	94	0		140	95
Medium	5	258	90	15	85	309	99
Medium	6	200	96	78	100	385	100
Medium	7	127	99	9	83	280	100
Medium	8	139	89	16	98	310	100
High	9	547	93	14	100	434	100
High	10	114	98	76	100	821	100
High	11	150	97	66	100	601	100
High	12	439	97	22	97	538	99

Discussion

As far as we are aware no-one has previously conducted multiple FECRTs on the same mob of animals (measured resistance in the same parasite population) on the same day. While full analysis of these data is yet to be conducted there are clearly some interesting results in this study.

FECRTs based only on FEC showed considerable variability within the individual flocks using a single equation. For example on Farm1 using equation 1, efficacies ranged from 84-96% and 9 of the 12 tests failed based on a failure threshold of <95% efficacy. On farm 2, using the same equation, efficacies varied from 92-99% and 2 of the 12 tests failed.

More obvious is the difference between formulae. On farm 1, efficacies using equation 1 varied from 84-96%, using equation 2 from 52-90% and using equation 3 from 85-96%. It might be more reassuring that, using equations 1 and 3, 9 of the 12 tests failed whereas with equation 2 all 12 tests failed. Thus at least there was a somewhat similar failure rate using all formulae. The large difference in calculated efficacies on this farm was due to a substantial rise in FEC of the untreated groups (all 12 of them) in the interval between samplings (mean FECs of the control groups at the pre and post-treatment samplings were 411 and 1430 epg respectively). Differences between equations were again obvious on Farm 2 where 5 of the 12 tests failed using equation 2, but using equation 3 none failed.

The values of the starting FEC appears to have had little influence on the test results on these 2 farms. Mean efficacies from the high, medium and low FEC groups are very similar and, without further analysis, there does not appear to be any more variability in any of the data. However,

all mean egg counts were 199 epg or greater, and there were no zero counts, so it may be that these numbers were all adequate to give a reasonable test result.

The variation in generic composition of faecal cultures was a surprise. Despite each culture being a pooled sample from 10 animals, and all cultures coming from a mob of lambs which had been grazing together for at least 2-4 months (farms 1 & 2 respectively), the proportion of each genus present varied considerably (Figures 1 and 2). On farm 2 the proportion of *Trichostrongylus* spp. in cultures varied from 13 to 96% and of *Cooperia* from 2-51%. Similar levels of variability occurred on Farm 1 (Figure 1). Clearly variations of these magnitudes are likely to substantially change the interpretation applied to culture results both for FECRTs and for more general diagnostics on-farm.

Efficacy by genus was quite variable in some cases but not in others. On Farm 1, efficacy against *Cooperia* varied from 0-87% but against *Trichostrongylus* from 95-100%. On Farm 2 efficacy against *Cooperia* ranged from 89-99%. It is not surprising that where the treatments worked effectively there was little variation in efficacy i.e., if a parasite is susceptible to the treatment, there should be none (or very few) present post-treatment, irrespective of the proportion in the pre-treatment (control) FECs. Where parasites are surviving treatment, however, wide variation in culture results is likely to result in even wider variation in efficacy estimates, as seen in these data.

Whether this variation in efficacy will result in a greater likelihood of an incorrect diagnosis is less clear. There was wide variation in efficacy by genus on Farm 1 and yet all tests failed against *Cooperia* and *Ostertagia* and all passed against *Trichostrongylus*. However, on Farm 2, 5 of 12 tests failed against *Cooperia* and 2 of 10 against *Ostertagia*.

It should be noted here that we have presented all the data, including those tests in which the FEC allocated to a genus (particularly *Ostertagia*) is low. McKenna (1996) has outlined the importance of adequate representation of genera in tests and has used a threshold level of 50 epg for inclusion of a genus in a test. Evidence to support this practice is seen on Farm 2 where two of the tests against *Ostertagia* failed in the presence of low initial counts (15 and 9 epg) against what would otherwise appear to be a susceptible population. Interestingly, despite low counts for *Ostertagia* on Farm 1, all tests failed, probably indicating a resistant population.

These results are intended only as a preliminary presentation to allow veterinarians to consider their implications. We have therefore endeavoured not to draw substantive conclusions at this point. Further analysis will be forthcoming and will hopefully be presented elsewhere in the near future. A general conclusion from these data might be that where efficacy due to resistance is low, a FECRT is quite reliable, and similarly where efficacy is high against a susceptible parasite (e.g., *Trichostrongylus* in these trials). Where test results fall into the efficacy ranges around 90-95%, variability is apparent and false-positive and false-negative tests undoubtedly occur. McKenna (1990) proposed a compromise category of 'suspected' resistance around these efficacy levels and these data would lend support for such a category.

However, what is less helpful is the test which returns a >95% efficacy when in fact the true value is lower. The only practical solution to this situation is for all farms(ers) to regard resistance as being 'present' and manage accordingly – certainly taking the position that 'its all right, I don't have resistance' on the basis of a one-off FECRT would seem to be a dangerous one.

References

Dash KM, Hall E, Barger IA. The role of arithmetic and geometric worm eggs counts in faecal egg count reduction test and in monitoring strategic drenching programs in sheep. Australian Veterinary Journal 65, 66-68, 1988

McKenna PB. The detection of anthelmintic resistance by the faecal egg count reduction test: An examination of some factors affecting performance and interpretation. New Zealand Veterinary Journal 38, 142-147, 1990

McKenna PB. Criteria for diagnosing anthelmintic resistance by the faecal egg count reduction test. New Zealand Veterinary Journal 42, 153-154, 1994

McKenna PB. Potential limitations of the undifferentiated faecal egg count reduction test for the detection of anthelmintic resistance in sheep. New Zealand Veterinary Journal 44, 73-75, 1996

Presidente PA. Methods for detection of resistance to anthelmintics. In: N. Anderson and P. J. Waller (ed). Resistance in nematodes to anthelmintic drugs, Pp 13-27. CSIRO Australia and Australian Wool Corporation Technical Publication, 1985