General Scientific Article

# Managing anthelmintic resistance – A role for undrenched adult ewes as a source of unselected parasites and in suppressing parasite populations?

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# Abstract

**AIMS:** To test the hypotheses that when undrenched adult ewes are rotationally grazed over pastures with drenched lambs the ewes can; i) function as a source of unselected parasites 'in refugia' capable of slowing the development of anthelmintic resistance, and ii) suppress the build-up of parasites resulting from the development of anthelmintic resistance.

**METHODS:** Firstly, the potential of undrenched adult ewes to slow the development of anthelmintic resistance, and to suppress parasite populations under differring levels of drench efficacy, was investigated using a simulation model. Secondly, a field trial with three replicates of each treatment, compared two grazing systems (Lambs only vs Lambs followed by Ewes) and two Drench types (albendaole to which resistance was present, faecal egg count reduction (FECR) = 57-59% and ivermectin + levamisole to which resistance was absent, FECR = 97-99%) in a factorial treatment structure. Parasite populations were monitored by faecal nematode egg counts (FEC), faecal larval cultures, pasture larval sampling and slaughter of tracer lambs. Animal performance was measured by liveweight, body condition score, dag score and fleece weights.

**RESULTS:** Model simulations indicated that unselected parasites cycling in the ewes could slow the development of resistance being selected by the lamb drenching and this could occur without a net increase in larval numbers on pasture. Further, as worm control in the lambs declined with increasing levels of drench resistance the ewes increasingly functioned as net removers of parasite larvae, effectively suppressing parasite populations.

In the field trial undrenched adult ewes contributed to pasture infestations of most parasite species, but not *Nematodirus*. Parasite species on pasture and infecting the lambs was changed when ewes were present but larval populations on pasture in the autumn where not greater. In the presence of anthelmintic resistance parasite populations were reduced when ewes grazed in rotation with lambs implicating the ewes as net removers of parasite challenge.

**CONCLUSIONS:** Undrenched adult ewes were a source of unselected genotypes, capable of slowing the development of anthelmintic resistance, in most, but not all, parasite species. Further, the potential of adult ewes to remove from pasture more parasite larvae than they contribute through faecal contamination indicates a potentially useful role in suppressing parasite populations, particularly when worm control in lambs in less effective as a result of anthelmintic resistance.



**KEYWORDS:** *anthelmintic resistance*, *refugia*, *ewes*, lambs, *larvae on pasture*


List of Abbreviations.

| | |
|---|---|
| FEC | faecal nematode egg count |
| EPG | eggs per g of wet faeces |
| FECR | faecal nematode egg count reduction |
| DM | dry matter |
| ALB | albendazole |
| IL | ivermectin + levamisole |
| L3 | third (infective) stage larva |

# Introduction

Anthelmintic resistance in nematode parasites of sheep and cattle is now common in New Zealand (Waghorn et al 2006a & b). In order to minimize the further development of resistance, and protect the efficacy of existing anthelmintic products, there is an urgent need to identify and implement farm management practices capable of reducing the selective advantage to resistant parasites. The concept of deliberately maintaining ‘refugia’ of unselected parasites to dilute resistant worms surviving treatment (van Wyk 2001) has become a cornerstone of resistance management principles and a number of approaches to creating refugia have been proposed. For example, treating animals with anthelmintic only on the basis of some indication of need, such as faecal nematode egg count (FEC) or dagginess (breech soiling), has found favour with some New Zealand farmers. An alternative is the routine or strategic treatment of a flock with a small proportion of animals deliberately left untreated (Michel 1985, Martin 1987, Leathwick et al 2006a). A third option is to rotationally or co-graze different stock classes (e.g., adult ewes with lambs) over the same pastures with only the lambs receiving anthelmintic treatments. While all of these options should, logically, create a reservoir of unselected parasite genotypes capable of slowing the development of resistance, there is still much research needed to clarify both their potential to do so, and the associated epidemiological and production costs, an important issue to the farmer considering their adoption.

A recent study by Leathwick et al (2006b) compared the effect of different drenching practices on the rate of development of benzimidazole resistance. An interesting observation in this trial was that despite significant levels of resistance developing in a number of parasite species over the trial period, clinical parasitism was never observed. In attempting to explain this apparent lack of ‘severe’ parasitism despite only moderate levels of drench efficacy it was hypothesised that rotationally grazing adult ewes with drenched lambs had functioned to suppress the build-up of parasite populations as drench efficacy declined. This would presumably depend on the adult ewes ingesting significantly more parasite larvae off pasture than they contributed through faecal contamination, thereby acting as net removers of parasite pasture contamination (Charleston 1986). If this hypothesis were correct, it would suggest that rotationally grazing undrenched adult ewes with drenched lambs might present a very attractive option to farmers attempting to manage both anthelmintic resistance and parasitism.

The present study was therefore undertaken to address the questions of whether undrenched adult ewes have the potential, when rotationally grazed over the same pastures as drenched lambs, to 1) slow the development of anthelmintic resistance by acting as a source of susceptible parasites ‘in refugia’? and 2) to suppress the build-up of parasite populations resulting from the development of anthelmintic resistance?

# Materials and Methods

This study was conducted in two parts, firstly using a simulation model of parasite dynamics and the development of anthelmintic resistance, and secondly by conducting a field trial.

### Model simulations

Simulations were run using the model previously described by Leathwick et al (1992, 1995). For all simulations variables were set to approximate the manner in which the previously referred to field trial (Leathwick et al 2006b) was run, i.e.;

1. lambs were born in August

2. ewes and lambs were set-stocked over all paddocks at a density of 18 ewes, plus their lambs, per hectare until weaning.
3. lambing percentage was 130%
4. lamb numbers were reduced in, December, February and May in line with lambs being progressively sold to slaughter, resulting in lamb numbers equivalent to 25% of ewe numbers being retained after May.
5. All lambs were given 5 oral drenches at 28 day intervals commencing at weaning in late November.

Two grazing scenarios were compared, one in which ewes and lambs grazed separate suites of paddocks after weaning (no cross contamination), and a second in which ewes followed lambs in grazing a common suite of paddocks (continuous cross contamination).

*Resistance development*

To investigate the influence of rotating undrenched ewes with drenched lambs on the development of drench resistance the two grazing scenarios outlined above were run, with an initial resistance gene frequency of $10^{-6}$, until drench efficacy (FEC reduction) dropped below 95%. The size of the reservoir of unselected parasites represented by the ewes was varied by setting the mean FEC of the ewe flock, over the period December to June, to levels between 50 and 200 epg. Years to reach an efficacy of <95% was taken as the measure of rate of resistance development.

*Worm population dynamics*

The influence of rotating undrenched ewes with drenched lambs on parasite infection in lambs (FEC) and infestation on pasture (Larvae/kg dry matter (DM)) was assessed. The two grazing scenarios were compared under three levels of anthelmintic resistance (drench efficacies of 99%, 70% and 40%). Further, in order to gauge the potential of ewes to act as net removers of larvae from pasture, ewe FECs were again varied from 50-200 epg.

## Field trial

A field trial was run on the Flock House research farm near Bulls in the Manawatu region from December 2004 to June 2006. The site was the same as that used previously by Leathwick et al (2006b) and had well developed resistance to benzimidazole anthelmintics in *Teladorsagia circumcincta*, *Trichostrongylus colubriformis* and *Nematodirus* spp, but all parasites were susceptible to both levamisole and macrocyclic lactone actives. Twelve farmlets were established in 3 replicate blocks of 4 treatments. Each farmlet consisted of a suite of either four or five paddocks (depending on treatment group) laid out in a randomised block structure so that paddocks within a farmlet were not adjacent to each other. Thus within each of the three main replicate blocks, paddocks were further ‘grouped’ into blocks of four adjacent paddocks which were randomly allocated to one of the four treatments. Treatments three and four each had an additional paddock outside of this layout.

Treatment structure was a 2 x 2 factorial design with two Stocking categories and two Drench types as follows;

| | |
|---|---|
| Trt. 1 - Lambs only | Drench Type I (resistance present) |
| Trt. 2 - Lambs only | Drench Type II (resistance absent) |
| Trt. 3 - Lambs + ewes | Drench Type I (resistance present) |
| Trt. 4 - Lambs + ewes | Drench Type II (resistance absent) |

Lamb-only farmlets consisted of 4 paddocks grazed by a single mob of lambs while lamb + ewe farmlets consisted of 5 paddocks grazed by a mob of lambs and a mob of ewes. In all cases the ewes were moved into a paddock on the day the lambs were moved out. Different numbers of paddocks were allocated to the different Stocking categories in order to maintain a three week spelling of pastures between grazing in all treatments. Overall stocking rates and the ewe to lamb ratio (on those farmlets with ewes) were balanced on calculated dry matter intake.

In November 2004 and 2005 approximately 600 lambs were purchased from an external source and transported to the research farm. On arrival, all lambs were treated orally with the manufacturer's recommended dose rate of a combination anthelmintic containing abamectin, levamisole and oxfendazole (Matrix, Ancare New Zealand Limited, Auckland New Zealand) to remove existing parasite burdens. All lambs were then shorn and weighed before being allocated to farmlet.

360 lambs were selected on the basis of uniformity of liveweight and randomly allocated to 12 groups of 30. Each of these groups was then randomly allocated to a farmlet and all 30 of these animals remained on that farmlet throughout the season (until June-July). These were the animals on which detailed measurements were made. Additional lambs were allocated to each farmlet on the basis of liveweight to meet the required pasture DM intake for that group. Mixed-age adult ewes were similarly allocated to treatment groups three and four. For example, in December 2004 Farmlet 1 contained 54 lambs (30 recorded animals + 24 to meet the intake requirements) while Farmlet 3 (which was approximately 20% larger) contained 36 lambs (30 + 6) plus 18 adult ewes. As lambs grew through the season and their intake increased, numbers of the non-recorded lambs, and some adult ewes, were reduced to maintain parity of DM intake across the treatments.

All lambs received five anthelmintic treatments, administered orally, at 28 day intervals commencing at weaning in December while adult ewes were never treated. Two anthelmintic products were used. For Drench Type I (resistance present) lambs were drenched with albendazole (ALB) (Albendazole, Ancare New Zealand Limited, Auckland, New Zealand) at 4.75 mg/kg whereas for Drench Type II (resistance absent) a levamisole + ivermectin (IL) combination was used (Erase MPC + Nilverm, Schering Plough Animal Health Limited, Wellington, New Zealand) at a dose rate of 7.53 mg/kg levamisole and 0.2 mg/kg ivermectin. Doses were calculated based on the heaviest lamb in each mob.

In July 2004, all lambs were removed from the trial and all farmlets were set-stocked with pregnant ewes for the winter. Equal stocking densities of ewes lambed on each farmlet where they remained, rearing their lambs, until weaning in December. They were then replaced with a new set of trial animals for the second year of the trial.

All experimental manipulation of animals was approved by the Crown Research Institutes' Animal Ethics Committe, Palmerston North, New Zealand.

**Parasitology**

*FEC*

Parasite populations in the ewes and lambs were monitored by FEC at approximately monthly intervals, with the lambs being sampled on the day they received each of their anthelmintic treatments. Faeces collected per rectum were placed in individually labelled plastic pottles and returned to the laboratory for processing. The number of strongylid and *Nematodirus* eggs present in a 2 g subsample was determined using a modified McMaster method in which each egg counted represented 50 eggs per g (epg) of wet faeces.

*FECR*

After the first (January) and fifth (April) drench treatments in year 1, and after the fifth (April) treatment in year 2, lambs were resampled for FEC 7-10 days after drenching in order to measure the efficacy of the treatment. Efficacy was estimated by comparing the pre- and post-treatment mean FEC for each group of lambs and calculating the percentage reduction in undifferentiated FEC.

*Larval cultures*

To monitor the generic composition of eggs passed by the ewes and lambs faecal larval cultures were carried out after every sampling for FEC. Faecal material surplus to that required for FEC was bulked within each farmlet, mixed with vermiculite and incubated at 20°C for at least 14 days. Infective third stage larvae (L3) were extracted by Baermannisation, concentrated in a water column by sedimentation at 5°C, and the generic composition of the first 100 L3s counted was recorded.

*Larvae on pasture*

Samples to estimate larval infestations on pasture were collected on four occasions (December, April, May and June) in year 1 and three occasions (December, May and June) in year 2. Samples were collected and processed separately from 2-3 paddocks within each farmlet on each occasion. Pasture was collected by hand ‘plucking’ herbage samples while walking zigzag transects across each paddock until 200-300g had been collected.

On return to the laboratory each herbage sample was soaked overnight in a large plastic funnel containing approximately 20 litres of water and 1-2 ml of surfactant. Larvae settling to the bottom of the funnel were decanted off and concentrated by sedimentation in a water column at 5°C. After storage at 5°C L3s were identified to genus and counted. The mean number of larvae per kg DM from each farmlet was taken as the data for analysis.

*Tracer lambs*

Each year 60 lambs, imported from another AgResearch farm, were selected on the basis of uniformity of liveweight and their date of birth (born September/October). After their arrival at Flock House they grazed predominantly on cattle pasture and were treated suppressively with anthelmintics to minimise their exposure to parasites. In May they were drenched free of parasites and held indoors for four weeks, before being moved onto the trial site in June. Five lambs were allocated to each farmlet on the basis of liveweight, and they grazed all the paddocks within their farmlet over a 20 day period. After a further 21 days indoors to allow maturation of ingested parasites all animals were slaughtered at a commercial abattoir and abomasa and small intestines recovered for worm counts.

Organs recovered at necropsy were repeatedly washed before 10% aliquots of the contents were passed over a 38 micron sieve and the retained material stored in 5% formalin. The number of adult worms present in half of each 10% sample (5% of the total) were identified to species and recorded. If fewer than 30 worms were present the remaining half of the 10% aliquot was also counted.

In 2005, no attempt was made to differentiate between small intestinal *Trichostrongylus* species (*colubriformis* and *vitrinus*) but in 2006 a subsample of male worms was individually extracted and examined under higher magnification. Species were differentiated on spicule morphology after Soulsby (1968).

*Productivity measures*

Lamb liveweights were recorded approximately monthly throughout while the degree of dagginess and body condition score of the lambs was assessed in January, February, May, June and July of 2005 and in January, March and May of 2006. In July 2005 lambs coming off the trial were shorn and fleeces weighed to give a measure of wool growth over the trial period. In 2006 the trial ended prematurely due to indications of clinical parasitism in some Treatment 1 groups (see below) and so no fleece weights were collected.

*Statistical analysis*

All data were analysed by ANOVA using a statistical model which included replicate, the main effects of Drench Type (ALB vs IL) and Stock Category (Lambs only vs Lambs + Ewes), and the Drench Type-by-Stock Category interaction. Because the experimental unit was the farmlet, the farmlet-within-replicate error term was used as the denominator in the F-test for treatment effects.

The proportions of different parasite genera in faecal cultures, the worm burdens of tracer lambs and the numbers of parasite larvae on pasture were all square root transformed before analysis. Data are presented as back-transformed means. For FECs, pasture larval counts in the autumn of each year, and faecal culture results, time was specified in the model prior to the comparison of drench and stock effects. For lamb liveweight gains, weaning weight was included as a covariate in the model.

## Results

### Model simulations

In the model simulations anthelmintic resistance developed more slowly when undrenched ewes grazed in rotation with drenched lambs than when drenched lambs grazed alone (Figure 1). As FEC of the ewes was increased, leading to an increase in their contribution to pasture contamination, resistance developed more slowly. However, this was not necessarily associated with an overall increase in the larval population on pasture or in lamb FECs. For example, when ewe FEC was set at 50 epg from weaning until July, the development of resistance was delayed while the peaks in autumn pasture infectivity and lamb FEC were reduced and this occurred over the full range of drench efficacies evaluated (outputs not shown).

This changed as ewe FEC was increased so that at a drench efficacy of 99% pasture larval infestations and lamb FECs were higher when ewes grazed with lambs than when lambs grazed alone (e.g., in June larvae on pasture numbered 267 for lambs alone and 337 for lambs + ewes, while lamb FECs were 1141 and 1474 epg respectively; Figure 2 a & d). However, as drench efficacies were decreased to 70% and 40% the ewes still functioned to reduce autumn parasite numbers on pasture and FECs in the lambs. This can be seen by comparing Figure 2 b) with 2 e) and 2 c) with 2 f) where even at a ewe FEC of 150 epg autumn pasture larval populations were reduced when ewes grazed with lambs.

## Field trial

*Drench efficacies*

Efficacy of the IL combination was high in all three of the FECR measured averaging, across the 6 farmlets, 99.2%, 99.6% and 97% for the three dates, although the reliability of these estimates was probably reduced due to low mean FECs in some pre-test samples (i.e. when mean FEC at the time of drenching was less than 50 epg). In contrast, efficacy of the ALB treatments averaged 57%, 59% and 57% for the three dates respectively. However, there was a consistent trend for the efficacy of ALB to be higher on farmlets where ewes were present than on those where lambs grazed alone; efficacies were 77% vs 37% (p=0.08) in January 2005; 80% vs 37% (p=0.072) in April 2005 and 74% vs 40% (p=0.108) in April 2006.

*FECs*

Average FEC (strongyle) in lambs was higher in 2005 when ewes grazed in rotation with lambs than when lambs grazed alone (240 epg vs 108 epg, p=0.016) but there was no difference attributable to Drench type (p=0.264) (Figure 3a). In contrast, in 2006 average FEC was higher in lambs drenched with ALB than in those drenched with IL (312 vs 143 epg, p=0.006) (Figure 3c). Further, although there was no effect of Stock category (p=0.732) in 2006 the Drench type by Stock category interaction was significant (p=0.025) (Figure 4) reflecting a substantial difference in lamb FEC between drenches when lambs grazed alone but a much smaller difference when ewes grazed in rotation with the lambs.

In 2005, average counts of *Nematodirus* eggs from the lambs was not effected by any of the treatments (Figure 3b) but in 2006 there were significantly more *Nematodirus* eggs recovered from lambs drenched with ALB than those drenched with IL (22 vs 3 epg, p<0.001) (Figure 3d).

Mean FEC in the undrenched adult ewes was not affected by the drench used to treat the lambs in either year (p= 0.879 & p=0.618 in 2005 and 2006 respectively) (Figure 5).

*Faecal cultures*

Faecal culture results indicated that parasite infection of the lambs was dominated by *T. circumcincta* and *Trichostrongylus* species (Figure 6). Both seasonal trends and differences due to treatment were evident in the generic composition of eggs passed in faeces. *T. circumcincta* tended to dominate egg output in the lambs early in the season and its abundance, relative to other parasites, declined with time (p<0.001 in both years). *Trichostrongylus* species contributed relatively little to egg output early in the season but came to dominate egg output in the autumn (p<0.001 in both years).

Drench type and/or Stock category influenced the relative abundance of all the main parasite genera. *T. circumcincta* made a larger contribution to egg output when IL was used as the anthelmintic than when ALB was used (38% vs 24%, p=0.036 in 2005; 43% vs 21%, p=0.002 in 2006). Also *T. circumcincta* tended to make a smaller contribution to lamb egg counts when ewes grazed with lambs than when lambs grazed alone (24% vs 37%, p=0.049 in 2005; 26% vs 36%, p=0.061 in 2006).

The proportion of *Trichostrongylus* larvae recovered from faecal cultures from lambs was not affected by grazing with ewes in either year (p=0.785 and p=0.866 in 2005 and 2006 respectively), but was significantly reduced, or approached significance, when lambs were drenched with IL compared with ALB (24% vs 39%, p=0.068 in 2005; 14% vs 40%, p<0.001 in 2006).

Recoveries of *Cooperia* larvae were not affected by drench type (p=0.495 & p=0.342 in 2005 and 2006 respectively) but were significantly higher when ewes grazed in rotation with lambs than when lambs grazed alone (14% vs 2%, p<0.001 in 2005; 20% vs 9%, p=0.011 in 2006).

Grazing with ewes reduced the proportion of *Nematodirus* larvae in faecal cultures in 2005 (2% vs 5%, p=0.032) and lambs drenched with ALB had a higher proportion of *Nematodirus* larvae than those drenched with IL in 2006 (10% vs 2%, p<0.001).

Faecal culture results indicated that parasite infection of the adult ewes in Treatments 3 and 4 were dominated by *Cooperia* species and *Oesophagostomum/Chabertia* (Figure 7). While there were some seasonal differences evident there were no differences due to which anthelmintic was used to treat the lambs.

*Larvae on pasture*

As expected, pasture plucks taken at the start of the trial in December 2004 showed no differences in larval populations between treatments. However, by autumn (April-June) there were significant treatment differences in all the main parasite genera and in total larval availability (Table 1). When ALB was used as the anthelmintic there were significantly more larvae in total on pasture than when IL was used. This was also seen in numbers of *Teladorsagia*, *Trichostrongylus* and *Nematodirus* larvae on pasture. Further, there were more *Cooperia* larvae on pastures when adult ewes grazed in rotation with lambs than when lambs grazed alone (Table 1). No other differences were significant although there was a trend, which approached significance, for there to be fewer larvae of some genera (*Trichostrongylus* and *Nematodirus*) on pastures when ewes were present.

In the following spring (December 2005) there were more *Teladorsagia* larvae on pastures where ALB had been used in lambs the following season and there was a trend (p=0.069) for there to be fewer *Teladorsagia* larvae on pastures where ewes had grazed with lambs. In other genera no differences were evident between treatments. In autumn 2006, there was a trend (p=0.064) for the total number of larvae present on pastures to be higher where ALB had been used. Also, there were significantly more *Nematodirus* larvae on pastures when lambs were drenched with ALB and fewer *Teladorsagia* larvae on pastures when ewes grazed in rotation with lambs (Table 1).

*Tracer lamb worm burdens*

In the autumn of both 2005 and 2006, tracer lambs grazing pastures in the autumn acquired higher total worm burdens where ALB, as compared to IL, had been used (Table 2). In 2005, there was also a significant effect of stock category with tracers grazed on lamb only farmlets having higher worm burdens. However, this effect was due largely to the influence of high worm counts in lambs grazing the ALB lamb-only farmlets resulting in the Drench type by Stock category interaction being significant (p=0.002, Table 2).

In both years the tracer lambs acquired higher burdens of *H. contortus*, *T. axei* and *Cooperia* species when grazing pastures previously grazed by ewes and lambs than by lambs alone (Table 2). In contrast, in 2005, they carried lower burdens of *T. circumcinta* when pastures had previously been grazed by ewes and lambs. Overall, in both years, small intestinal

*Trichostrongylus* species were not affected by Stock category but numbers were higher when lambs were drenched with ALB rather than IL. However, in 2006 when a subsample of these were identified to species, there were significantly fewer *T. colubriformis* on pastures previously grazed by ewes and lambs than by lambs alone (means of 444 & 8505, p=0.002). No other differences due to Drench type were detected in the worm burdens of the tracer lambs.

*Lamb Liveweight gain*

Liveweight gain in the lambs showed considerable variability over time as reflected in a plot of their liveweights (Figure 8). Growth rates were highest in January 2005 (150 g/day) and December 2005 (138 g/day), and lowest in March 2006 (-35 g/day). This variable performance tended to reflect the seasonal pattern of pasture growth and quality on the farm with maximum pasture growth occurring in late spring to early summer (December – January) and pastures drying off, with an associated loss of quality, over the February – April period.

The influence of lamb weaning weight on subsequent liveweight gain approached significance in both years (p= 0.085 and p= 0.064 in 2005 & 2006 respectively). Averaged over each season, lambs grazed in rotation with undrenched adult ewes grew significantly faster than lambs grazed alone (87 vs 65 g/day, p<0.001, in 2005 and 80 vs 57 g/day, p=0.004, in 2006). Lambs drenched with IL grew significantly faster than those drenched with ALB which was not fully effective due to resistance (83 vs 69 g/day, p=0.003, in 2005 and 78 vs 59 g/day, p= 0.012, in 2006). This resulted in lamb liveweight differentials of 2.6 kg (p=0.003) and 3.4 kg (p=0.006) in June of 2005 and 2006 respectively.

*Condition and dag scores*

At the start of the season (Dec-Jan), in both years, there were no differences between treatments in body condition score of the lambs (Table 3), which was to be expected given that the lambs were sourced externally as a uniform group of animals and were randomly allocated to farmlet based on liveweight.

By May 2005, and March 2006, there were significant differences between Drench types and Stocking categories and these persisted for the remainder of each season (Table 3). In all cases lambs grazed with ewes had higher condition scores than those grazed alone and lambs drenched with ALB had lower condition scores than those drenched with IL.

In January of both years there was no difference between treatments in the degree of dagginess (breech soiling) of the lambs reflecting the fact that all lambs were crutched and shorn prior to coming onto the trial in December.

However, by May 2005 lambs grazed in rotation with adult ewes had fewer dags than lambs grazed alone (Table 4) and this trend continued until July. In June and July 2005, there was also a significant difference in dagginess associated with Drench type with lambs drenched with IL having more dags than those drenched with ALB. In July 2005, the Drench-by-Stock interaction was also significant (p=0.028, Table 4). This reflected the fact that when ALB was used, lambs grazed alone had more dags whereas when IL was used lambs grazed with ewes had only a slightly higher dag score.

In March and May of 2006, there was no effect of Stock category on dag score in the lambs but lambs drenched with ALB had significantly more dags than those drenched with IL (Table 4).

*Fleece weights*

In July 2005 lambs drenched with IL had heavier fleeces than those drenched with ALB (2.37 kg vs 2.15 kg, p=0.014) and lambs grazed with adult ewes had heavier fleeces than those grazed alone (2.38 kg vs 2.15 kg, p=0.010).

## Discussion

This study was carried out principally to address the questions of whether undrenched adult ewes have the potential, when rotationally grazed over the same pastures as drenched lambs, to 1) slow the development of anthelmintic resistance by acting as a source of susceptible parasites 'in refugia'? and 2) suppress the size of parasite populations building up through the development of anthelmintic resistance?

There is clear evidence from the faecal cultures that the adult ewes were cycling a range of parasite species and, by virtue of their not being treated with anthelmintic, were therefore acting as a source of unselected worms. This is supported by other data which indicated that the ewes were contributing to pasture contamination. For example, FEC in the lambs was on average higher in 2005 where ewes were present which is perhaps to be expected given that, unlike the lamb-only treatments, a proportion of the grazing animals were not treated with anthelmintic. This is particularly evident in Figure 3a where the effectiveness of repeated whole flock treatments with IL can be seen in the continuing low FECs of the lamb-only Stock category. Further, there is evidence from both the pasture sampling and tracer lamb worm burdens that the ewes were contributing to autumn pasture infestations of at least some parasite species (*H. contortus*, *T. axei* and *Cooperia* spp.). This finding is consistent with the study of Brunsdon (1970) who found that the dominant parasites in adult ewes throughout much of the year were *H. contortus*, *T. axei*, *Cooperia curticei* and *T. circumcincta*.

Contributing to pasture contamination is, by default, a requirement of the concept of 'refugia'. For unselected parasites to play a role in diluting the survivors of anthelmintic treatments, and thereby delaying the build-up of resistance, they must produce offspring and contribute to future generations of parasites. In the field trial there was evidence that the ewes did contribute to pasture infestation and therefore, logically, they could be expected to slow the development of anthelmintic resistance. Not surprisingly, the modelling supports this view, indicating that where ewes are making a contribution to pasture contamination, which has the opportunity to mix with contamination from drenched lambs, a reduction in the rate of development of anthelmintic resistance can be expected (Figure 1). Also, as expected, the model indicates that the greater the ewe's contribution to pasture contamination (i.e. the higher their FEC) the more the development of resistance will be slowed.

However, it is also clear from the field trial that the species composition of parasite eggs passed by the ewes was different to that passed by the lambs. While infection in the lambs was dominated by *T. circumcincta* and *Trichostrongylus* species, that in the ewes was dominated by *Cooperia* and large intestinal species (*Oesophagostomum* / *Chabertia*). This in itself does not negate a possible role for the ewes as a useful source of unselected parasites as long as they are cycling sufficient numbers of those species being selected by drenching the lambs. It does, however, warn against a simplistic view of the role of adult ewes as a source of both pasture contamination and refugia based, for example, on FECs alone. Perhaps the best example of this is *Nematodirus* which consistently occurred in the lamb FECs and on pasture (Tables 1 & 2) but the eggs of which were almost never recovered from ewes. Older sheep generally develop a firm resistance to infection with this parasite (Brunsdon 1967) and its eggs are seldom seen in numbers from adult ewes (Brunsdon 1964, McKenna 1981). It can therefore be concluded that adult ewes are likely to be a very poor source of 'refugia' for these species.

The modelling suggested that contribution to pasture contamination by adult ewes does not automatically imply higher overall levels of pasture infectivity. In the simulations, when ewe FECs averaged 50 epg or less there was a net reduction in larval populations on pasture in the autumn despite the contribution from the undrenched ewes, and this occurred at all levels of drench efficacy. This presumably reflects the fact that the ewes, because of their well developed resistance to infection, ingest and kill more parasite larvae than they contribute through faecal contamination (Charleston 1986). Further, in the model, as worm control in the lambs became less effective due to anthelmintic resistance the ewes continued to function as net removers of larvae, suppressing the worm population compared with equivalent lamb only systems (Figure 2). This occurred even when pasture contamination from the ewes was increased through higher FECs. It appears then that, in the model, as pastures become increasingly contaminated with parasite larvae derived from the lambs, the ingestion and removal of larvae by the adult ewes continues to exceed the additional contamination they contribute.

In the field trial, despite clear indications of the ewes contributing to pasture contamination, there was no overall increase in the number of larvae on pasture in the autumn as measured by pasture plucks and tracer lambs. There were, however, differences in the species composition of the larval populations in that grazing with ewes resulted in more larvae of *Cooperia*, *H. contortus* and *T. axei*, but fewer of *T. circumcincta* and *T. colubriformis*. In 2005, lambs grazed by themselves had lower FECs than those grazed with ewes which suggests an increased larval challenge, although this was not detected in the pasture sampling. It could be relevant here that prior to the trial commencing the site was grazed solely with drenched cattle for five months which is likely to have reduced the initial parasite challenge to the lambs. This may have artifically inflated the importance of larval contamination derived from the ewes in the early phases of the experiment. Whatever the cause, in both years of the field trial, the ewes contributed unselected parasites, of most species, into the larval population without causing any substantial increase in total pasture contamination.

Further, comparison of the two ALB Treatments demonstrated the ability of adult ewes to suppress parasite populations in the presence of anthelmintic resistance. This was most obvious in 2005 when worm burdens of the tracer lambs, which reflect pasture larval infestations, were higher on the lamb-only farmlets. For total worm burden, *T. circumcinta* and small intestinal *Trichostrongylus* spp. burdens the Stock category-by-Drench type interactions were significant or nearly so ($p=0.002$, $p=0.007$ & $p=0.064$ respectively). A similar effect was evident in 2006 in the lamb FECs (Figure 4) where again the interaction was significant ($p=0.025$). These results indicate that when lambs grazed alone and were treated with a drench which was only partially effective due to resistance, the buildup of larvae on pasture in autumn and subsequent infection in the lambs (FEC) was greater than when ewes grazed in rotation with the lambs. Thus the results of the field trial mirrored the outputs from the modelling and both supported our initial hypothesis that, in the presence of anthelmintic resistance, grazing pastures with undrenched adult ewes can usefully suppress parasite population growth.

Lambs grazed in rotation with adult ewes performed better than those grazed alone as indicated by liveweight gains and body condition scores in both years and by dag score and fleece weights in 2005. Given that lambs grazed with ewes had higher FECs in 2005, and that no reduction in number of larvae on pasture as a result of ewe grazing was recorded in either year, it seems unlikely that these responses were due to parasite effects. Never-the-less, grazing in rotation with ewes did alter the species composition of the infection in the lambs and so, it remains possible that differences in the pathogenicity of the infection may have contributed to the observed differences in performance, despite the increase in FEC. However, other workers have also found that lamb growth rates differ between grazing regimes in a manner unrelated to FEC suggesting that differences in performance relate to the complexities of pasture composition, growth and diet selection (Marley et al 2006) and this is the likely cause here as well.

The data also allows estimates of the production costs of anthelmintic resistance to be made. Overall, in this trial, lamb liveweight gain was poor due to trying to grow lambs under conditions of pasture quantity and quality which were not suitable. Therefore the 14-19 g/day difference in liveweight gain measured between Drench types is likely to be a very conservative estimate of the cost of resistance. These values represent a 19-24% reduction in growth rate, which if applied to lambs growing at a respectable 200 g/day, would equate to losses due to anthelmintic resistance of 38-48 g/day. Other production losses were also evident with fleeces from ALB treated lambs being 170g lighter. Reductions in condition score were measured and in 2006 an increase on dags. It is not clear why in 2005 lambs drenched with IL had significantly more dags than those drenched with ALB.

While these data reflect a long-term chronic impact of parasites on lamb performance as a result of anthelmintic resistance, they may still underestimate the full potential cost. In 2006, this trial was ended prematurely when groups of Treatment 1 (ALB, Lambs only) lambs began to show signs of clinical parasitism. In one group a monitor FEC taken 7 days after the fourth drench was higher than the pre-drench count and exceeded the 1500 epg threshold for intervention on ethical grounds. At that point all Treatment 1 groups were treated with levamisole and the trial was terminated for all groups three weeks later after the scheduled fifth treatment. In this case careful monitoring and strict rules around animal welfare prevented significant parasitism from developing. Had this not been the case it seems certain that the impact of escalating worm challenge as a result of anthelmintic resistance would have been much more severe than indicated in these data.

In this trial undrenched adult sheep were a source of pasture contamination for most parasite species, and indeed they must be if they are to function as a useful source of unselected parasites ‘in refugia’. However, it is also well established that the potential of adult sheep to contaminate pastures is much less than that of undrenched lambs. For example, trials in New Zealand showed that pastures grazed by undrenched 2-tooth wethers or adult ewes over summer (December – February) had autumn larval infestations reduced by 93-98% compared with pastures grazed by undrenched lambs (Brunsdon 1980). Of course, where lamb drenching is highly effective at suppressing contamination of pastures the contribution from the ewe is more easily measured (e.g., in Treatment 4 of this trial), and this has been the basis for suggesting that adult ewes are a substantial source of infection in lambs (Familton 1991 – non peer-reviewed, Stafford et al 1994) and should, therefore, be the focus of anthelmintic treatments in autumn and winter (Familton et al 1995). Unfortunately, an obvious outcome of the routine treatment of all stock classes has been the development of anthelmintic resistance.

The present study has shown that undrenched ewes, grazed in rotation with drenched lambs, offer a potentially useful source of unselected parasites for most, but not all, parasite species. It has also shown that when parasite control in lambs is not highly effective, in this case as a result of anthelmintic resistance, ewes can function to usefully suppress the growth of parasite populations. Combined with the fact that lambs grazed with ewes consistently performed better than equivalent lambs grazed alone, these results suggest that undrenched adult ewes are worthy of further consideration as a source of unselected parasites in 'refugia' when developing strategies to manage anthelmintic resistance.

## Acknowledgements

Annette Litherland gave advice on stock management, Lawrie McMurtry assisted with lab work and John Koolard advised on statistical matters. An industry mentor group consisting of Dave Smith, Chris Ridland, James Falloon, Mark Gilmour, Tony Rhodes, Alex Vlassoff, Bill Pomroy and Dave West made invaluable contributions to the design and execution of the trial. Stewart Bisset and Dave West made helpful comments on an earlier manuscript. The work was funded by Meat and Wool New Zealand under contract number 03AR/44.

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**Figure 1** – Time, expressed relative to drenched lambs grazing alone, to reach a drench efficacy of <95% in model simulations when adult ewes rotationally grazed after drenched lambs and mean ewe FEC was varied between 50 and 200 epg.

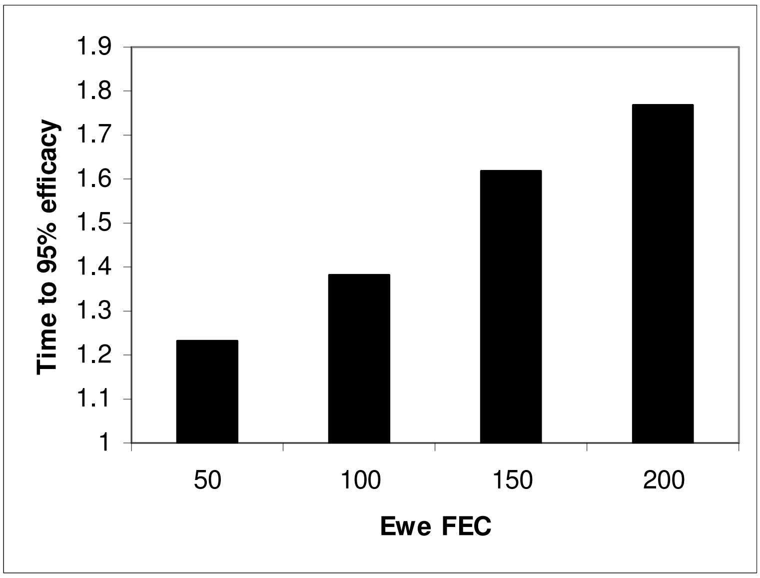
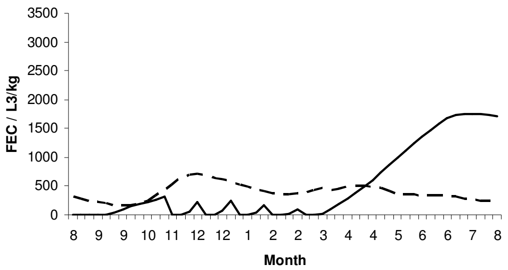


**Figure 2** – Output from model simulations comparing grazing scenarios where lambs graze in a rotation with (a, b & c) or separately from (d, e, & f) undrenched adult ewes after weaning. FEC of the undrenched ewes was set at a constant 150 epg from December until July. Lambs were administered 5 anthelmintic treatments at 28 day intervals starting at weaning and drench efficacies were varied from 99% (a & d), 70% (b & e) to 40% (c & f). Data are lamb faecal egg counts (epg - solid lines) and pasture larval populations (L3/kg herbage – dashed lines).

a)

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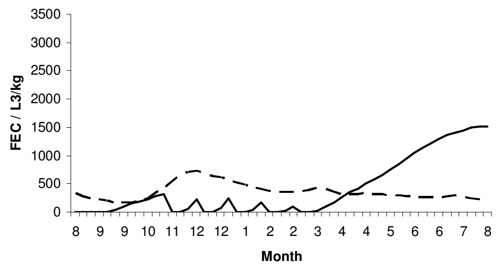


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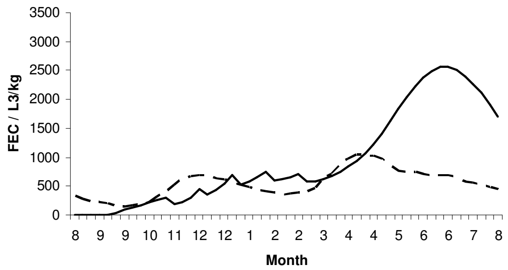


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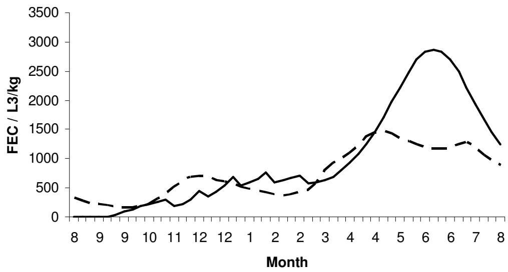


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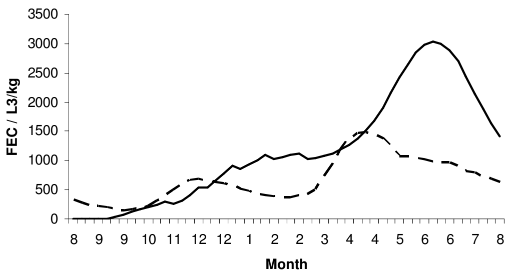


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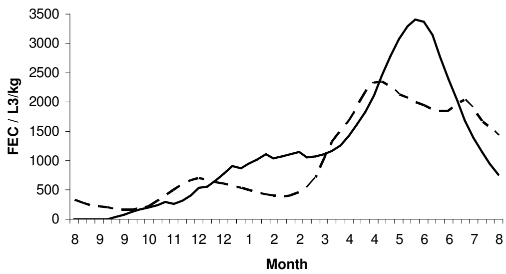
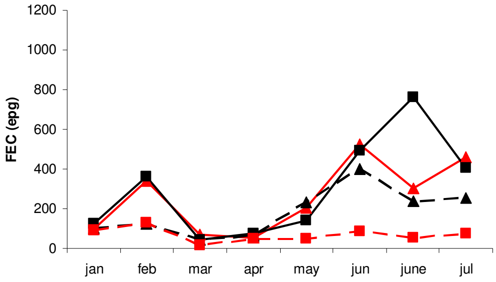
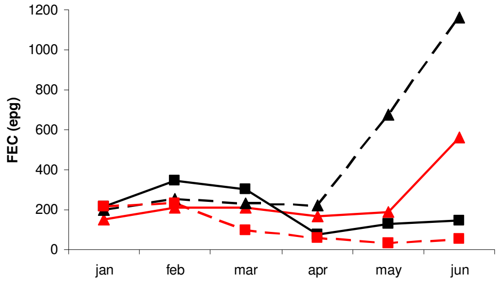


**Figure 3** – Faecal nematode egg counts (FEC) from lambs administered 5 drenches at 28 day intervals in 2005 (a & b) and 2006 (c & d) divided into strongylid (a & c) and *Nematodirus* (b & d) counts. Solid lines = undrenched adult ewes grazed in rotation with drenched lambs; Dashed lines = drenched lambs grazed alone; ▲= lambs drenched with albendazole; ■ = lambs drenched with a combination of ivermectin and levamisole.

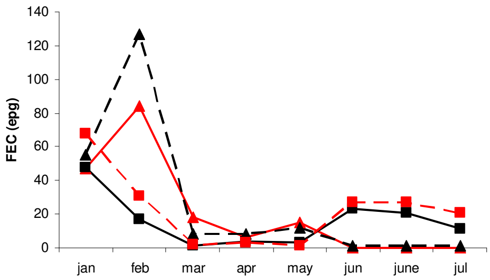
a)




c)




b)

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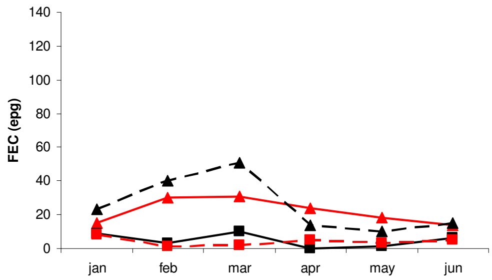


**Figure 4** – Interactions plot of the mean FEC (strongyle) over a six month period (January – June) in 2006 from mobs of lambs which received 5 drenches at 28 day intervals with either albendazole (▲) or a combination of ivermectin and levamisole (■) and which either grazed alone or in rotation with a mob of undrenched adult ewes.

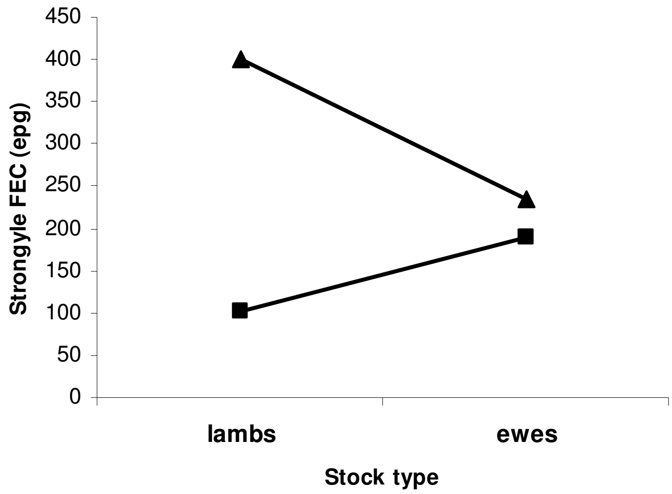
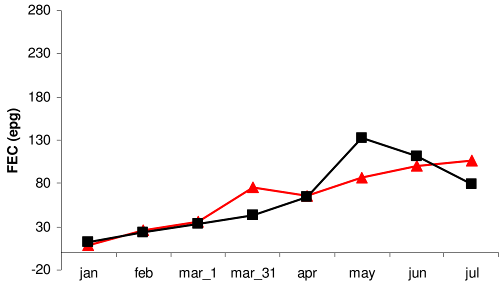


**Figure 5** – Faecal nematode egg counts (FEC) from undrenched mixed-age ewes in 2005 (a) and 2006 (b). Ewes were rotationally grazed with lambs which received 5 drenches at 28 day intervals with either albendazole (▲) or with a combination of ivermectin and levamisole (■).

a)




b)

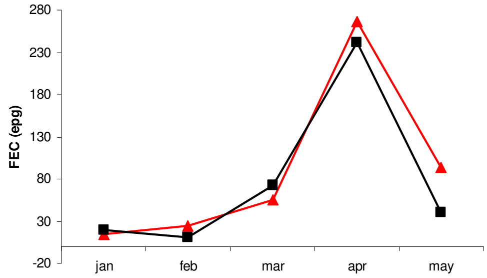


**Figure 6** – Percentages of third stage larvae of different parasite genera recovered from faecal cultures collected from lambs in 2005 (a, b, c, d) and 2006 (e, f, g, h). Parasite genera are *Teladorsagia* (a & e), *Trichostrongylus* (b & f), *Cooperia* (c & g) and *Nematodirus* (d & h). Blue & Red = lambs grazed alone; White & Grey = lambs grazed in rotation with adult ewes; Blue & White = lambs drenched with albendazole; Red & Grey = lambs drenched with ivermectin + levamisole.

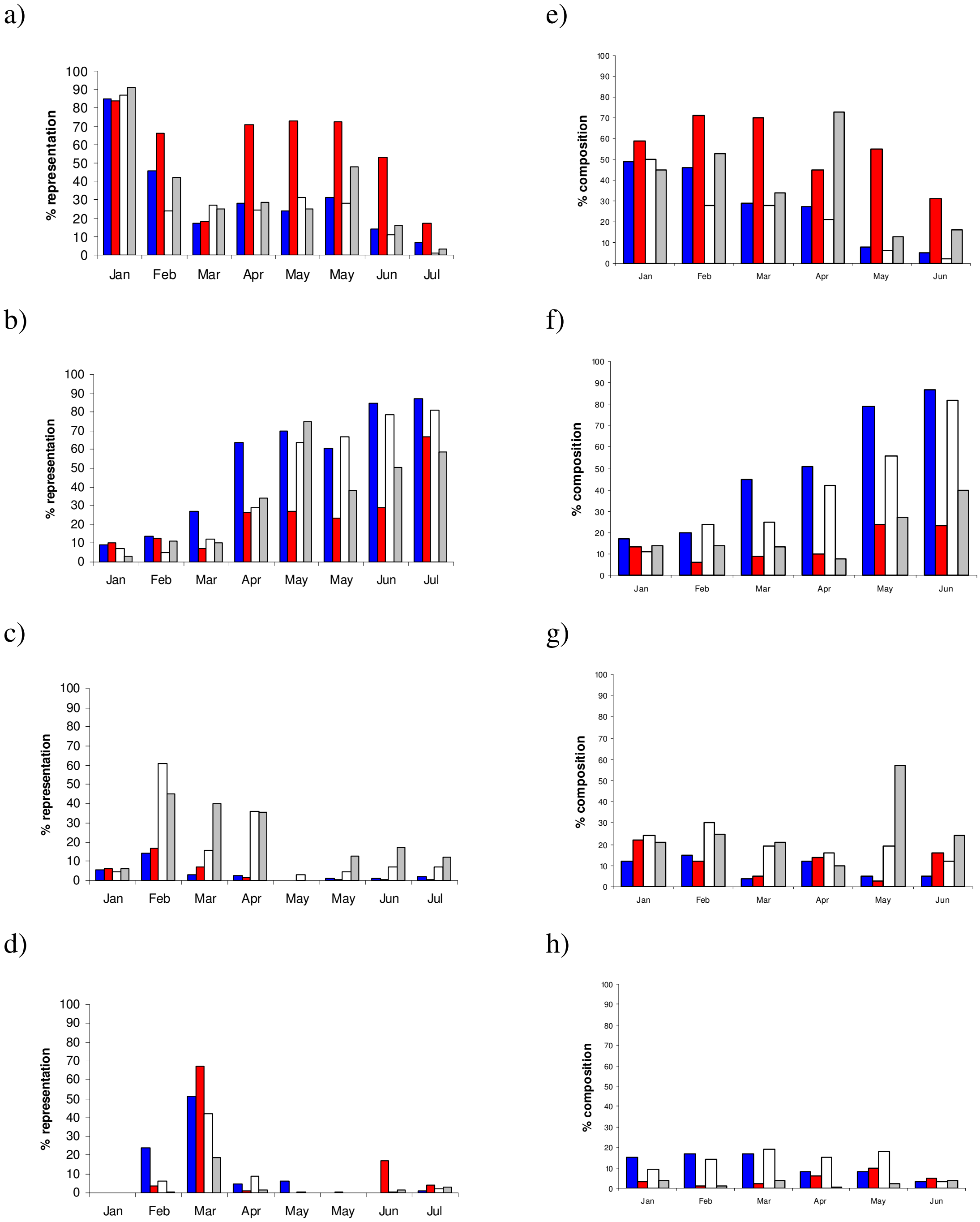


**Figure 7** – Percentages of third stage larvae of different parasite genera recovered from faecal cultures collected from undrenched adult ewes in 2005 (a, b, c, d) and 2006 (e, f, g, h). Parasite genera are *Teladorsagia* (a & e), *Trichostrongylus* (b & f), *Cooperia* (c & g) and *Oesophogostermum*/*Chabertia* (d & h). Blue = lambs drenched with albendazole; Red = lambs drenched with ivermectin + levamisole.

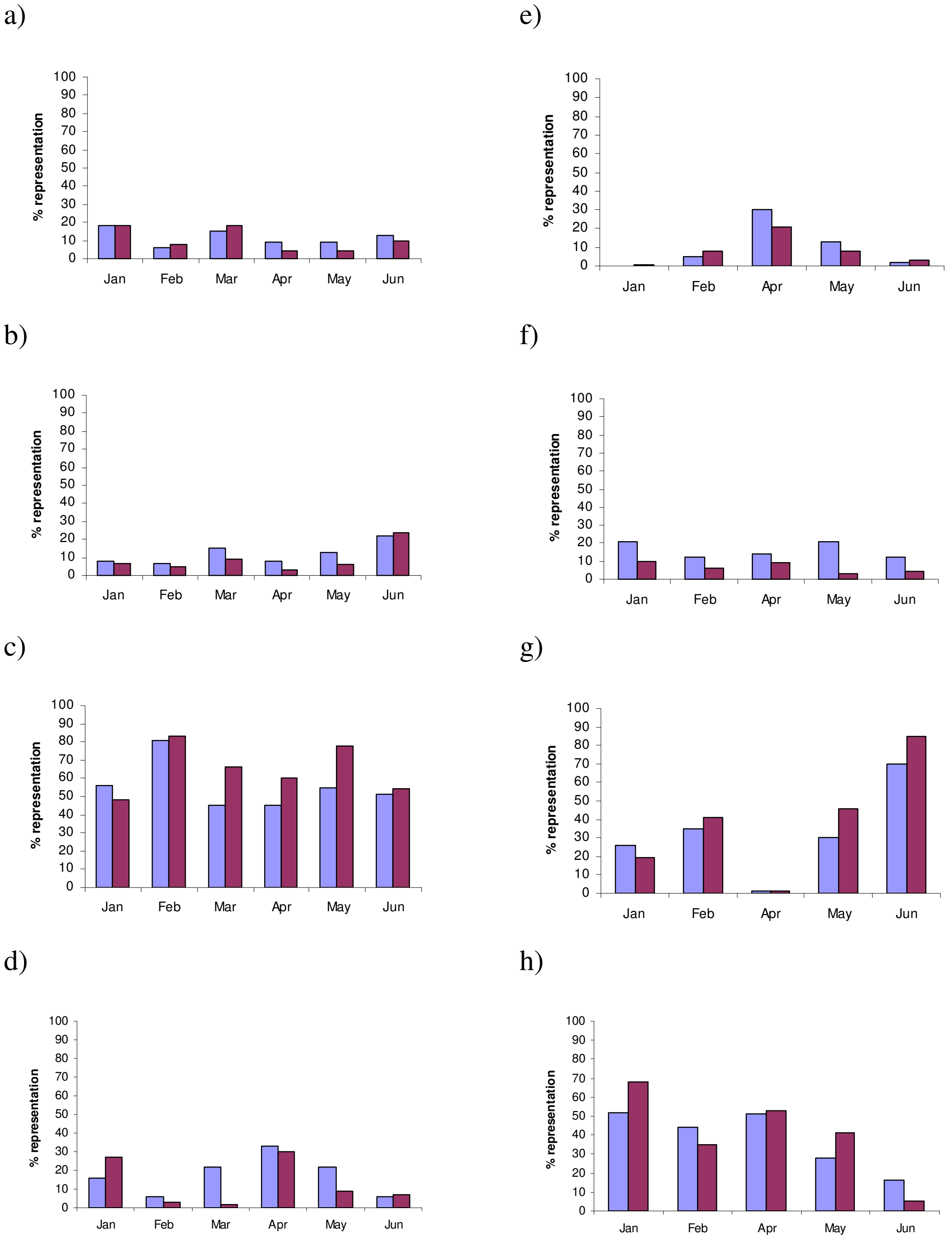
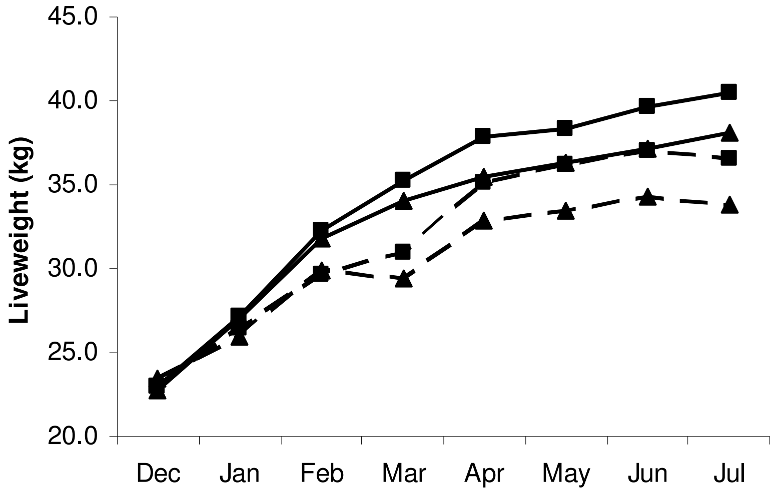


**Figure 8** – Mean liveweight of lambs administered 5 drenches at 28 day intervals in 2005 (a) and 2006 (b). Solid lines = undrenched adult ewes grazed in rotation with drenched lambs; Dashed lines = drenched lambs grazed alone; ▲= lambs drenched with albendazole; ■ = lambs drenched with a combination of ivermectin and levamisole.

a)




b)

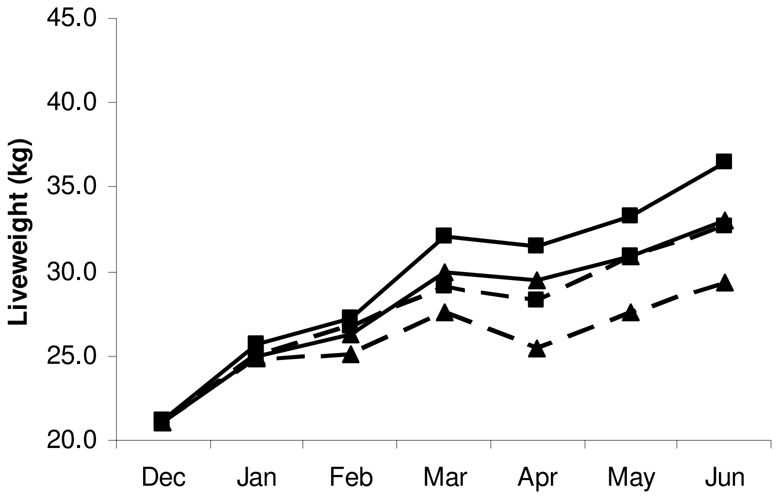


Table 1 – Pasture larval infestations (L3/Kg DM) of *Trichostrongylus*, *Teladorsagia*, *Cooperia* and *Nematodirus* in spring and autumn from pastures grazed either by lambs alone or by lambs and adult ewes in which lambs were drenched with either albendazole (to which resistance was present) or ivermectin + levamisole (to which resistance was absent).

| Date | Drench | Stock Category | *Tri* | *Tel* | *Coop* | *Nem* | Total |
|---|---|---|---|---|---|---|---|
| Dec-04 | Alb | Lambs only | 22 | 63 | 0 | 150 | 228 |
| | Alb | Ewes+Lambs | 27 | 93 | 0 | 80 | 177 |
| | IL | Lambs only | 40 | 184 | 0 | 182 | 311 |
| | IL | Ewes+Lambs | 87 | 167 | 5 | 202 | 378 |
| | | P; Drench | 0.257 | 0.103 | Nt* | 0.350 | 0.423 |
| | | P; Stock | 0.442 | 0.908 | Nt | 0.756 | 0.999 |
| | | P; Drench*Stock | 0.532 | 0.661 | Nt | 0.574 | 0.736 |
| Apr-Jun 05 | Alb | Lambs only | 242 | 338 | 12 | 554 | 1167 |
| | Alb | Ewes+Lambs | 90 | 149 | 56 | 316 | 643 |
| | IL | Lambs only | 57 | 60 | 1 | 158 | 289 |
| | IL | Ewes+Lambs | 45 | 70 | 31 | 41 | 219 |
| | | P; Drench | 0.018 | 0.004 | 0.091 | 0.004 | 0.003 |
| | | P; Stock | 0.091 | 0.163 | 0.008 | 0.067 | 0.138 |
| | | P; Drench*Stock | 0.189 | 0.099 | 0.661 | 0.943 | 0.350 |
| Dec-05 | Alb | Lambs only | 72 | 527 | 2 | 15 | 619 |
| | Alb | Ewes+Lambs | 94 | 429 | 7 | 45 | 584 |
| | IL | Lambs only | 94 | 397 | 10 | 23 | 600 |
| | IL | Ewes+Lambs | 56 | 258 | 2 | 7 | 318 |
| | | P; Drench | 0.747 | 0.031 | Nt | 0.362 | 0.121 |
| | | P; Stock | 0.737 | 0.069 | Nt | 0.632 | 0.093 |
| | | P; Drench*Stock | 0.235 | 0.714 | Nt | 0.181 | 0.163 |
| May-Jun 06 | Alb | Lambs only | 328 | 590 | 2 | 740 | 1756 |
| | Alb | Ewes+Lambs | 266 | 317 | 26 | 331 | 980 |
| | IL | Lambs only | 250 | 449 | 23 | 72 | 824 |
| | IL | Ewes+Lambs | 196 | 270 | 18 | 61 | 566 |
| | | P; Drench | 0.431 | 0.390 | 0.283 | 0.013 | 0.064 |
| | | P; Stock | 0.543 | 0.050 | 0.185 | 0.319 | 0.148 |
| | | P; Drench*Stock | 0.981 | 0.753 | 0.099 | 0.386 | 0.572 |

*Tel* = Teladorsagia
*Tri* = Trichostrongylus
*Coop* = Cooperia
*Nem* = Nematodirus
Alb = albendazole
IL = ivermectin + levamisole
* Nt – not tested because of large number of zero values

Table 2 – Worm burdens (back-transformed after square root transformation) from tracer lambs after grazing pastures previously grazed either by lambs alone or by lambs and adult ewes in which lambs were drenched with either albendazole (to which resistance was present) or ivermectin + levamisole (to which resistance was absent).

| Date | Drench | Stock Category | *T.cir* | *H.con* | *T.axe* | *Tri* | *Coo* | *Nem* | Total |
|---|---|---|---|---|---|---|---|---|---|
| Jun-05 | Alb | Lambs only | 4434 | 2 | 0 | 1518 | 8 | 7122 | 14175 |
| | Alb | Ewes+Lambs | 702 | 34 | 277 | 261 | 84 | 3070 | 4640 |
| | IL | Lambs only | 1318 | 0 | 1 | 79 | 3 | 3142 | 4621 |
| | IL | Ewes+Lambs | 1345 | 63 | 141 | 161 | 235 | 3646 | 5861 |
| | | P; Drench | 0.092 | 0.772 | 0.484 | 0.029 | 0.241 | 0.232 | 0.010 |
| | | P; Stock | 0.008 | 0.002 | 0.003 | 0.155 | 0.002 | 0.211 | 0.010 |
| | | P; Drench*Stock | 0.007 | 0.210 | 0.388 | 0.064 | 0.107 | 0.107 | 0.002 |
| Jun 06 | Alb | Lambs only | 5503 | 6 | 421 | 14879 | 209 | 2261 | 24838 |
| | Alb | Ewes+Lambs | 6418 | 264 | 1757 | 7795 | 781 | 2265 | 21786 |
| | IL | Lambs only | 7641 | 80 | 116 | 437 | 93 | 2647 | 11321 |
| | IL | Ewes+Lambs | 5048 | 415 | 861 | 1110 | 665 | 2720 | 11470 |
| | | P; Drench | 0.795 | 0.171 | 0.070 | 0.003 | 0.290 | 0.680 | 0.015 |
| | | P; Stock | 0.521 | 0.010 | 0.008 | 0.537 | 0.003 | 0.971 | 0.744 |
| | | P; Drench*Stock | 0.196 | 0.745 | 0.791 | 0.206 | 0.677 | 0.974 | 0.708 |

*T.cir* = Teladorsagia circumcincta
*H.con* = Haemonchus contortus
*T.axe* = Trichostrongylus axei
*Tri* = small intestinal Trichostrongylus
*Coop* = Cooperia species
*Nem* = Nematodirus species
Alb = albendazole
IL = ivermectin + levamisole

Table 3 – Body condition scores (1 = poor condition, 5 = fat to overweight) for lambs either grazed alone or in rotation with adult ewes and which were drenched with either albendazole (to which resistance was present) or ivermectin + levamisole (to which resistance was absent).

| Date | Drench | Stock Category | Jan | Feb | May | Jun | Jul |
|---|---|---|---|---|---|---|---|
| 2005 | Alb | Lambs only | 1.56 | 1.28 | 1.85 | 2.10 | 2.17 |
| | Alb | Ewes+Lambs | 1.63 | 1.31 | 2.10 | 2.28 | 2.38 |
| | IL | Lambs only | 1.56 | 1.34 | 2.04 | 2.30 | 2.36 |
| | IL | Ewes+Lambs | 1.69 | 1.44 | 2.23 | 2.45 | 2.48 |
| | | P; Drench | 0.669 | 0.276 | 0.042 | 0.019 | 0.029 |
| | | P; Stock | 0.152 | 0.490 | 0.014 | 0.026 | 0.016 |
| | | P; Drench*Stock | 0.704 | 0.703 | 0.624 | 0.798 | 0.401 |
| | | | Jan | Mar | May | | |
| 2006 | Alb | Lambs only | 1.16 | 1.00 | 1.71 | | |
| | Alb | Ewes+Lambs | 1.14 | 1.32 | 1.97 | | |
| | IL | Lambs only | 1.12 | 1.25 | 2.00 | | |
| | IL | Ewes+Lambs | 1.21 | 1.52 | 2.09 | | |
| | | P; Drench | 0.701 | 0.006 | 0.010 | | |
| | | P; Stock | 0.304 | 0.002 | 0.020 | | |
| | | P; Drench*Stock | 0.132 | 0.656 | 0.190 | | |

Alb = albendazole
IL = ivermectin + levamisole

Table 4 – Dag scores (0 = no dags, 4 = heavily soiled around breech and legs) for lambs either grazed alone or in rotation with adult ewes and which were drenched with either albendazole (to which resistance was present) or ivermectin + levamisole (to which resistance was absent).

| Date | Drench | Stock Category | Jan | Feb | May | Jun | Jul |
|---|---|---|---|---|---|---|---|
| 2005 | Alb | Lambs only | 0.16 | 1.32 | 0.99 | 1.56 | 1.84 |
| | Alb | Ewes+Lambs | 0.16 | 1.20 | 0.37 | 1.05 | 1.25 |
| | IL | Lambs only | 0.20 | 1.33 | 1.24 | 2.95 | 3.58 |
| | IL | Ewes+Lambs | 0.24 | 0.89 | 0.74 | 2.70 | 3.65 |
| | | P; Drench | 0.240 | 0.531 | 0.219 | 0.000 | 0.000 |
| | | P; Stock | 0.741 | 0.245 | 0.046 | 0.002 | 0.068 |
| | | P; Drench*Stock | 0.724 | 0.504 | 0.811 | 0.105 | 0.028 |
| | | | Jan | Mar | May | | |
| 2006 | Alb | Lambs only | 0.88 | 2.05 | 2.21 | | |
| | Alb | Ewes+Lambs | 0.82 | 1.61 | 2.24 | | |
| | IL | Lambs only | 0.60 | 1.05 | 0.83 | | |
| | IL | Ewes+Lambs | 0.77 | 1.31 | 1.43 | | |
| | | P; Drench | 0.381 | 0.000 | 0.001 | | |
| | | P; Stock | 0.752 | 0.157 | 0.105 | | |
| | | P; Drench*Stock | 0.546 | 0.001 | 0.138 | | |

Alb = albendazole
IL = ivermectin + levamisole