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NO ADAPTATION OF ROUNDWORMS TO RESISTANT HOSTS

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SUMMARY
A genetically diverse population of *Haemonchus contortus* was serially passaged through Merino hosts bred for either increased or decreased resistance to the parasite. After 30 parasite generations, the resulting nematode sub-populations were compared with each other and with the foundation population, by infecting lines of the selected sheep and measuring the resulting faecal egg counts (FECs) and packed cell volume declines (PCVDs). There was no evidence from either FECs or PCVDs, that parasites passaged through resistant hosts had changed in response to host selection.
Keywords: Genetic resistance, sheep, immunity, *Haemonchus contortus*, adaptation.

INTRODUCTION
With growing concern about the sustainability of chemical methods for controlling roundworms, breeding sheep for resistance to roundworms has been the focus of considerable research effort over the past decade. Although genetic progress towards increased resistance can be relatively rapid compared with genetic changes in other traits, improving resistance is still a long-term process (Eady *et al*. 1997). Reservations have been expressed that worms have a much higher reproduction rate and shorter generation interval than sheep and are likely to adapt to resistant hosts under field conditions. In this paper we report an investigation of whether *Haemonchus contortus* will adapt to Merinos that have been bred for increased resistance to that parasite.

MATERIALS AND METHODS
Sub-populations of *H. contortus*, which were serially passaged for 30 generations through Merino lines bred for either increased (IRH) or decreased (DRH) resistance, were compared on the basis of their egg output and level of anaemia in their hosts. The sheep selection lines were described by Woolaston and Piper (1996) and the establishment of the parasite sub-populations, designed to be genetically diverse, was described by Woolaston *et al*. (1992). Host animals for passaging were drawn from the sheep selection lines, chosen at random from those males having egg counts within one phenotypic standard deviation of their respective line means when tested at 5-6 months of age. For each parasite generation, two rams from each line were used. Most rams were used for two successive cycles, so that a total of 34 rams from each line were represented. The average age of the rams at time of infection was 385 days, the same in each line (range 163-850 days). One week prior to infection, host animals were dosed with ivermectin at twice the manufacturer's recommended rate then housed on a slatted floor and fed a pelleted ration. A single dose of larvae was administered orally in equal numbers to each host line. Dose rates averaged 19500 infective larvae per head, but varied slightly to allow for the age of host animals (range 15000-25000). Approximately 22-23 days after infection, faeces were collected, and incubated. Faecal egg counts were recorded at the time of

collection. Larvae were recovered from the faecal cultures, pooled from the two replicate hosts, then used to reinfect the next two rams from each respective host line.

To test for changes in the parasites, larvae representing the foundation population (G0) were thawed from liquid nitrogen and passaged once through two immunosuppressed wethers to increase the numbers. At the same time, 30th generation larvae from each of the two test strains (IRH and DRH parasite lines) were also passaged through immunosuppressed wethers. Infective larvae from the three parasite populations were then administered orally to two flocks at pasture: one comprising 236 mixed sex yearlings from the three *Haemonchus* selection lines (IRH, DRH and their unselected controls, CH); the other comprising 419 mixed-age (two to six years) pregnant ewes from the same lines. Animals were treated with an effective anthelmintic one week prior to challenge and allocated at random within selection line to one of the three parasite populations. The yearling flock was infected with 10000 L3 larvae per head and the ewe flock with 6000 L3 larvae per head. At the time of challenge, packed cell volumes were determined. Yearlings were an average age of 413 days when infected, and the ewe flock was infected 58 days before the start of lambing. The average lambing dates were similar in all three lines, as were the number of ewes that failed to lamb. Faecal egg counts (FECs) and packed cell volume declines (PCVDs) were determined in all animals five weeks after infection.

RESULTS

The mean FEC in passaging hosts from the IRH line was 6614 eggs per gram (epg) and from the DRH line was 21318 epg. A cube-root transformation was found to be most appropriate for analysis of FEC data, and when applied, this between-line difference was highly significant. The linear regression of worm generation also affected FEC in the passaging hosts (Table 1), but there was no significant interaction between line and treatment, nor was there any effect of age of ram or variation in numbers of larvae given. Faecal egg count increased significantly with worm generation at an average rate 0.29±0.09 $epg^{0.33}$ per generation, but the increase was similar in both IRH hosts (0.26±0.12 $epg^{0.33}$ per generation) and DRH hosts (0.32±0.12 $epg^{0.33}$ per generation).

Table 1. Summary of analysis of variance of cube-root transformed faecal egg counts (epg) in ram hosts used to passage *H. contortus*

Source of Variation	df	Mean Square	P
Host line	1	2219.38	<0.0001
Worm generation (linear)	1	727.75	<0.01
Line x generation	1	9.17	NS
Error	115	65.89	

When the parasite strains were tested, mean FECs (untransformed) of the yearlings were 845, 6981 and 12638 epg for IRH, CH and DRH lines, respectively. The respective means in the breeding ewes were 772, 2187 and 2595 epg. These differences were highly significant in both flocks (Table 2) and reflected in average PCVDs (Figure 1), although overall, the packed cell volumes of most breeding ewes actually increased during the experiment.

Table 2. Summary of analysis of variance in faecal egg counts (FEC, epg$^{0.33}$) and packed cell volume decline (PCVD, %) in yearlings and breeding ewes

	Yearlings					Breeding ewes				
Source of		FEC		PCVD			FEC		PCVD	
Variation	df	Mean sq	P	Mean sq	P	df	Mean sq	P	Mean sq	P
Sheep line	2	4634.3	<0.001	512.36	<0.001	2	1494.1	<0.001	37.35	<0.05
Parasite strain	2	111.0	NS	103.23	<0.05	2	49.9	NS	128.82	<0.001
Sex	1	678.8	<0.001	25.58	NS					
Sheep x parasite	4	33.2	NS	43.76	NS	4	6.3	NS	11.49	NS
Error	226	54.4		28.97		409	37.9		11.43	

Parasite strain had no effect on five week FECs in either flock, but significantly affected PCVD, with the strain passaged through DRH sheep causing the greatest decline in haematocrit in most cases (Figure 1). There was no interaction between sheep line and parasite strain for FEC or PCVD in either flock.

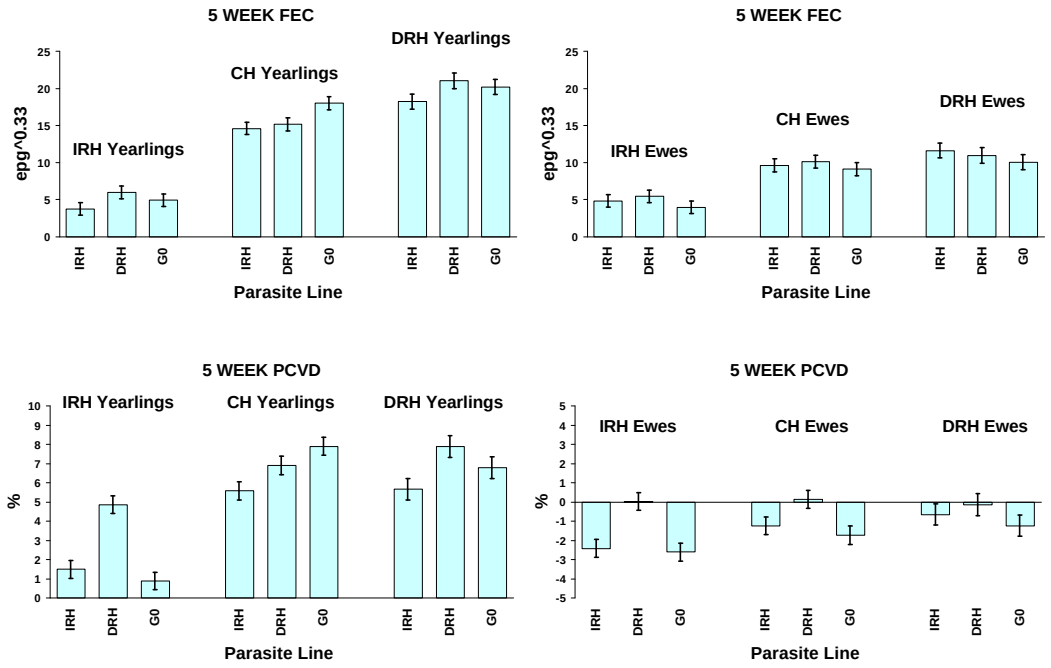

Figure 1. Mean faecal egg count (FEC) and packed cell volume decline (PCVD) in mixed-sex yearlings and breeding ewes. Bars show standard errors.

DISCUSSION

These results show that passaging parasites experimentally through sheep increased their egg output at about 3 weeks, as seen by the positive linear effect of generation in passaging hosts. This was also apparent in differences observed between the strains in FEC from data collected in this experiment three weeks after infection of the 30th generation, but not included in this paper. Because the parasite generation interval was deliberately kept low to increase the number of generations selected within the time constraints of the project, this probably resulted in some selection pressure being applied to younger worm age at first egg laying. However, by five weeks, these differences had disappeared. It is unclear why the strain that had been passaged through DRH hosts had a greater effect on haematocrit than the other strains, even though FECs were not differentially affected.

A more important finding is that although the parasites apparently responded to selection for a short generation interval, they did not respond to hosts with genetically enhanced resistance. In neither flock was there was any evidence that parasites bred in IRH hosts, were relatively more pathogenic towards animals in the IRH line. This supports our conclusion after the 14th generation (Woolaston *et al*. 1992). The persistence of genetic resistance beyond weaning has again been demonstrated, this time in both rams and ewes. Faecal egg counts in the IRH passaging hosts were only about 30% of those in the DRH hosts, and in the breeding ewes, FECs in IRH ewes were only 35% of those in CH ewes. In mixed-sex yearlings, FECs in the IRH line were only 12% of those in the CH line.

The 30 generations of worm selection in this experiment were probably equivalent to about 15 years in the field. All rams contributed equally to the ensuing generation of parasites, so selection pressure in both lines would have been somewhat greater than that encountered at pasture where animals with higher egg counts contribute most to the following parasite generation. Nevertheless, the selection intensity was very much lower than used in experiments designed to induce drug resistance in *H. contortus* populations. For example, Egerton *et al*. (1988) showed that seven generations of selection with ivermectin dosed to produce 95% efficacy, caused a marked increase in the resistance of *H. contortus* to that drug. Thus a longer experiment with more resistant animals, might still see the emergence of resistant worms. Nevertheless, to put our results into context, improvements in resistance of the magnitude seen in the IRH line are large enough to cause a significant reduction in mortalities of weaners from nematodiasis (Eady 2001), yet apparently do not induce a detectable genetic response in the parasite.

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