HOW EARLY IS RESISTANCE TO NEMATODE PARASITES EXPRESSED IN MERINO LAMBS BRED FOR RESISTANCE TO *HAEMONCHUS CONTORTUS*?

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SUMMARY

The CSIRO *Haemonchus* selection flock was used to determine the earliest age at which differences in faecal egg count (FEC) between the increased resistance (IRH), decreased resistance (DRH) and control (CH) lines become apparent. FEC's were measured on three occasions in young lambs aged between 30 and 97 days, when grazing pastures contaminated with mixed strongyle species. A significant difference in $FEC^{0.33}$ between the IRH line and the other lines emerged at the second sampling, when the average age of the lambs was 65 days (back-transformed means of the three lines were 128, 201 and 290 eggs per gram, respectively). Not only were the IRH lambs more resistant at this early age, but they also began to express resistance earlier than the other lines. This should reduce levels of pasture contamination and possibly drenching requirements.

Keywords: Genetic resistance, nematodes, immunity, *Haemonchus contortus,* lambs

INTRODUCTION

Age effects on resistance to the common nematode parasites of sheep have been well documented (eg. Manton *et al.* 1962; Smith *et al.* 1985), with resistance increasing up to 12 months of age. The early months of a lamb's life represent a critical period in the epidemiology of a parasitic nematode population. The increase in larvae numbers on pasture following the peri-parturient rise in lactating ewes occurs at a time when if ingested by naive hosts, these larvae should develop to sexual maturity without impediment (Wakelin 1986).

Genetic selection is an effective method of altering the resistance of Merino sheep to nematode parasites (Gray 1991; Windon 1996; Woolaston & Baker 1996). Heritability of resistance (based on faecal egg count, FEC) to *Haemonchus contortus* is around 0.2 to 0.3 (Albers *et al.* 1987; Woolaston & Piper 1996; Eady *et al.* 1996). Consequently, the CSIRO *Haemonchus* selection lines have achieved considerable divergence in the primary trait, which is FEC following artificial challenge with *H. contortus* at an average age of 25 weeks (Woolaston & Piper 1996). Selection at this age also confers favourable changes in resistance later in life (Woolaston 1992) which has potential epidemiological advantages. Further advantages may accrue if the progeny of resistant sheep show enhanced resistance prior to selection age. Here we report a study to determine the earliest age at which differences in FEC become apparent in lines of unweaned grazing lambs selected for increased or decreased resistance to *H. contortus.*

MATERIALS AND METHODS

The sheep were from the 1992 drop CSIRO *H. contortus* selection flock comprising lambs with either increased resistance (IRH, n=91), decreased resistance (DRH, n=74) or unselected (CH, n=97). The flock is maintained at Armidale on the Northern Tablelands of NSW. Full details of the flock structure, management and selection procedure can be found in Woolaston and Piper (1996). Pastures were improved, comprising chiefly phalaris, fescue, white clover and subterrerean clover. To compensate for pasture shortage during drought conditions prior to the currrent experiment, the flock was supplemented with wheat grain from late June until mid-October when adequate spring rain fell.

Lambing commenced on September 9 and lasted for 47 days. Four and a half weeks into lambing, all lambs greater than a day old and their mothers were drifted to a holding paddock managed to be relatively free of nematode larvae. The average age of lambs drifted was 15.3 days. Three weeks later, these ewes and lambs were moved to a contaminated paddock. The ewes with lambs born after the drift, joined the flock in this paddock at the completion of lambing, approximately 2_ weeks later. Larvae numbers on the contaminated pasture were estimated two weeks before the sheep were introduced, using the technique of Heath & Major (1968).

Faecal samples were collected from lambs held on the contaminated pasture, on three occasions, three weeks apart. The average ages (±SD) of lambs at the three samplings were 43.6±5.7, 64.7±6.9 and 84.3±7.3 days respectively, and age distributions were similar in all three lines. Samples could not be obtained from 17 lambs on the first occasion without causing undue stress on the lambs, and 3 lambs were not sampled on the second occasion. At the first sampling, no attempt was made to collect faecal samples from the younger lambs which had not yet joined the flock from the lambing plots, as they were less than 28 days old and assumed not to have produced positive FEC's. FEC's were determined using the modified McMaster technique with a lower level of detection of 100 eggs per gram (epg). On each sampling occasion, bulked faecal cultures were prepared for larval species differentiation. For the first sampling, a single bulk sample was made across all selection lines, while for the second and third collections, samples were bulked within selection lines.

Although statistical tests were always applied to cube root transformed data, untransformed mean egg counts are reported in the text unless otherwise stated. A repeatability least squares model was fitted which included line, animal within line, sampling time and the line by time interaction. Fixed effects of birth rearing rank, dam age and sex were fitted but found to be non significant and were removed from further analyses. Transformed data from each sampling day were also analysed separately, fitting line and within-line regression on age at sampling.

RESULTS

The average (±SD) FEC at the first sampling was 239±458 epg; at the second sampling was 364±410 epg; and at the third sampling was 1,881 ± 1,409 epg. The proportions of lambs with zero egg counts were 48.9 %, 17.4 % and 1.2 %, respectively.

The repeatability analysis revealed a highly significant ($P<0.01$) interaction between line and sampling time and the estimate of repeatability of $FEC^{0.33}$ was 0.23±0.04. At the second and third samplings, IRH lambs had significantly lower FEC's than CH lambs (Table 1) and at the third sampling they were also significantly lower than DRH lambs. At the first two samplings, there was a significant tendency for older lambs to have higher FEC's (Table 1). By the third sampling, FEC's of IRH and CH lambs declined with age, but not of DRH lambs. At the subsequent standard artificial challenge, all line means differed significantly (back-transformed means 6,381, 11,098 and 22,592 epg for the IRH, CH and DRH lines, respectively, $P<0.001$).

Table 1. Back-transformed mean FEC (epg) and effect of age (r, epg$^{0.33}$/day)

	1st sampling		2nd sampling		3rd sampling	
	FEC	r	FEC	r	FEC	r
IRH	47.9^{a}	0.33±0.07	128.2^{a}	0.06±0.05	10,08.9^{a}	-0.19±0.05
CH	41.6^{a}	0.42±0.06	290.4^{b}	0.17±0.05	18,58.9^{b}	-0.12±0.05
DRH	52.3^{a}	0.40±0.06	200.8^{ab}	0.17±0.04	18,06.4^{b}	0.02±0.04

Means in the same column with different superscripts are significantly different ($P<0.05$)

From the pasture sampling, of the average of 1,461 L3 larvae present per kg dry matter, 14 % were *H. contortus*, 71 % were *T. colubriformis* and 14 % were *Ostertagia circumcincta*. Results of faecal cultures (Table 2) confirm that *T. colubriformis* and *O. circumcincta* were the predominant species.

Table 2. Percentage of larval species from faecal cultures prepared at each sampling

Worm species (%)	1st sampling (Pooled over lines)	2nd sampling IRH	2nd sampling CH	2nd sampling DRH	3rd sampling IRH	3rd sampling CH	3rd sampling DRH
H. contortus	0	11	6	15	10	11	18
T. colubriformis	1	10	5	4	72	66	35
O. circumcincta	99	79	89	81	18	23	47

DISCUSSION

On average, IRH lambs were beginning to show resistance when the mean age was 9 weeks; earlier than the other two lines. At their second sampling, the FEC of IRH lambs was lower and within that line, there was no effect of age. In the other two lines, older lambs still tended to have higher FEC's at the second sampling. Within-line effects of age may be at least partly due to younger animals simply having insufficient time to ingest larvae and for these larvae to reach sexual maturity and begin laying eggs, particularly at the first sampling. This is supported by the fact that at the third sampling, the IRH and CH regressions were reversed in sign and the older animals had, on average, lower FEC's. Thus older animals appear to have reached a point where an effective immune response was taking place; a phenomenon not found in the DRH line.

At the first faecal sampling the mean lamb age was 43.6 days and the youngest lambs were just 30 days old. Previous studies of sheep under similar conditions showed that suckling lambs began to graze at 21 days of age, when they consumed 10g per day digestible organic matter from pasture, or less than 3 % of their total daily intake (Langlands & Donald 1975). Pasture sampling indicated a dominance of *T. colubriformis*, but this was not evident in the faecal cultures until the third sampling. However, the worm eggs present at the first two samplings would have come mainly from larvae ingested in the lambing plots and holding paddock, which were not pasture sampled.

The present work is in general agreement with the pen trials using unselected sheep of McClure *et al* (1998), except that IRH lambs had developed partial immunity earlier; by 65 days of age. Although the flock was selected for resistance to *H. contortus* under artificial conditions, it is evident that the immunising pasture infections consisted mainly of other nematode species, as *T. colubriformis* and *O. circumcincta* were predominant in the faecal cultures. The substantial "cross resistance" of IRH sheep to other nematode species has been found previously (eg. Woolaston 1992).

These findings suggest that pasture contamination will be considerably less with genetically resistant animals. Not only do peri-parturient ewes of the IRH line reduce pasture contamination relative to unselected sheep (Woolaston 1992), but unweaned lambs are also better able to resist any worms that are present. Watson & Gill (1991) showed that weaning can retard the development of immunological responses to internal parasites. It appears that IRH lambs are able to mount an effective immune response to stronglye parasites earlier than DRH and CH lambs and well before weaning, which may put them at an advantage during this time of additional stress.

ACKNOWLEDGEMENTS

We thank Dr L.R. Piper for his valued input into the statistical analysis. Financial assistance for this work was provided by Australia's woolgrowers through AWRAP.

REFERENCES

Albers, G.A.A., Gray, G.D., Piper, L.R., Barker, J.S.F., Le Jambre, L.F. and Barger, I.A. (1987) *Int. J. Parasitol.* **17**:1355

Eady, S.J., Woolaston, R.R., Mortimer, S.I., Lewer, R.P., Raadsma, H.W., Swan, A.A. and Ponzoni, R.W. (1996) *Aust. J. Agric. Res.* **47**:895

Gray, G.D. (1991) In "Breeding for Disease Resistance in Farm Animals", p.139-161, editors R.F.E.Axford and J.B. Owens, CAB International, Wallingford

Heath, D.D. and Major, G.W. (1968) *J. Helmin.* **42**:299

Langlands, J.P. and Donald, G.E. (1975) *Anim. Prod.* **21**:175

Manton, V.J.A., Peacock, R., Poynter, D., Silverman, P.H. and Terry, R.J. (1962) *Res. vet. Sci.* **3**:308

McClure, S.J., Emery, D.L., Bendixsen, T. and Davey, R.J. (1998) *Int. J. Parasitol.* **28**:739

Smith, W.D., Jackson, F., Jackson, E. and Williams, J. (1985) *J. Comp. Path.* **95**:235

Wakelin, D. (1986) *Proc. 6th Int. Cong. Parasitol.* p. 549-557, Brisbane, Qld Australia

Watson, D.L. and Gill, H. S. (1991) *Res. Vet. Sci.* **51**: 128

Windon, R.G. (1996) *Vet.Immunol. Immunopath.* **54**:245

Woolaston, R.R. (1992) *Int. J. Parasitol.* **22**:947

Woolaston, R.R. and Baker, R.L. (1996) *Int. J. Parasitol.* **26**:845

Woolaston, R.R. and Piper, L.R. (1996) *Anim. Sci.* **62**:451