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Developments in breeding Perendale sheep for resistance or susceptibility to internal nematode parasites

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ABSTRACT

Selective mating of Perendale sheep for increased or reduced susceptibility as lambs to an experimental challenge with *Haemonchus contortus* commenced in 1986. Four years later changes were assessed by monitoring faecal nematode egg count (FEC) in ewes and their progeny.

In the absence of drenching, mature flock ewes were given 7000 infective larvae of *H. contortus* and 3000 larvae of *Trichostrongylus colubriformis* in mid May 1990 after mating. FEC were monitored at regular intervals during pregnancy and 4 times during the periparturient period. Ewe and ram lamb progeny were faecal sampled on 5 occasions between weaning (November 1990) and May 1991. Lambs were drenched at most sampling dates. All stock were grazed across pasture contaminated previously with mixed parasite populations.

Adult ewes selected as lambs for Resistance (low FEC) shed fewer eggs than Susceptible (high FEC) line ewes throughout pregnancy, 2 weeks pre- and 3, 6 and 9 weeks post - parturition. At the periparturient peak, approximately 3 weeks after lambing, Low FEC line ewes maintained FEC which were only 25% of those in the High FEC line.

Lambs from the Low FEC line had significantly lower FEC than their High counterparts on 3 of 5 sample dates ($P<0.01$). At the final sample date (30 April 1991), there was a 10-fold difference in mean FEC between extreme sire groups. Bulked faecal cultures demonstrated that *Haemonchus* and *Trichostrongylus* were the dominant genera.

Keywords Nematode, parasites, genetic, resistance, ewes, lambs, Perendale, pregnancy, parturition, faecal egg count, *Haemonchus contortus, Trichostrongylus colubriformis.*

INTRODUCTION

With approximately 2,800 million dollars of sheep export earnings threatened by anthelmintic resistant internal nematode parasites and increasing client demand to apply fewer animal remedies, producers are looking to alternatives for chemical control. In New Zealand, research programmes have investigated aspects of natural host resistance in Romney and Perendale sheep. During the last decade research has concentrated on estimating heritabilities and developing resistant and susceptible lines of sheep using regimes with either natural mixed infection (Romney) or controlled experimental challenge (Perendale) (Watson *et al.*, 1986; Baker *et al.*, 1991; Bisset *et al.*, 1991; Watson and Hosking 1991).

The present trial was completed to resolve 2 questions. Firstly, would ewes selected for low or high FEC follow the same pattern of resistance or susceptibility during their breeding years? Secondly, would progeny of animals selected following experimental challenge with *H. contortus* be equally responsive to natural mixed infection? These data along with seasonal and periparturient patterns of FEC for their dams are reported herein.

MATERIALS AND METHODS

Flock History/Establishment

Perendale lambs born in 1984 and 1985 from the Ministry of Agriculture and Fisheries flock at Wairakei were screened for extreme high and low FEC. The flock was transferred to Ruakura in 1984-85 and first experimental matings began in 1986. These formed the basis of an on-going selection programme investigating resistance to *Haemonchus contortus*. The protocol was simple. Each lamb was vaccinated with 250 infective (L3) larvae kg^{-1} liveweight after weaning, generally between 12 and 16 weeks of age. Infections were abbreviated with anthelmintic 63 days later and each lamb was challenged 14 days later with 350 L3 larvae kg^{-1} liveweight. Selection of ram and ewe replacements has been based solely on FEC ranking. These were collected when FEC was predicted to peak, 5-7 weeks after each infection.

At least 3 sires have been used in the Resistant (R) and Susceptible (S) line annually. Each year, ewes were single-sire mated. Lambs were tagged at birth. Full lambing and production records including identification, sex, sire, dam, birthdate, birth rearing rank, weaning weight (WWT) and date, and various body and fleece weights (FWT) have been taken.

Since 1990 the resistant line has been further separated into lines developing early (ER) or late (LR) resistance. By definition, ER lambs are those which will tend to be immune in that they failed to demonstrate a FEC above 600-900 epg after both challenges at a very young age (4-6 months) while LR are those which acquire immunity in the 'normal' pattern (9-12 months).

Trial Protocol

Difficulties with parasite cultures made it impossible to test the Perendale lambs born in 1990 under the adopted protocol. Therefore the decision was made to determine their levels of resistance/susceptibility following natural mixed nematode infection.

Sixty-eight ewes involved in the trial came from 1986 or 1987 lambings. After mating, ewes of all lines were grazed as one mob. On 15 May 1990 each ewe was given 7,000 *H. contortus* and 3,000 *Trichostrongylus colubriformis* infective larvae *per os* by stomach tube in tap water. FEC were determined at weekly intervals throughout the winter (May-August). The ewes were separated into lambing groups approximately 2 weeks prior to predicted onset of lambing. Ewes from the 2 main lambing cycles were faecal sampled 2 weeks pre- and 3, 6 and 9 weeks post-lambing.

Fifty ram and sixty-four ewe lambs were grazed across land used to pasture the flock over the previous 4 years. Lambs were weaned in November 1990. At this time rectal faecal samples were taken from each lamb and they were drenched. FEC was monitored and lambs were drenched at intervals between weaning and April 1991. All lambs were given anthelmintic treatments at weaning, 14 January, 18 February, and 30 April following sampling. Both sexes grazed as one mob until they were sampled and drenched in February. Thereafter, they ran as 2 groups.

Liveweights were recorded 5 times from weaning to 30 April 1991. Lambs were shorn in April and fleece weights recorded.

Venous blood samples taken in April were used to determine serum gamma glutamyl-transferase (GGT) levels in all lambs to assess liver damage.

Bulked faecal cultures were used to differentiate nematode composition at regular intervals during the study.

Least squares mean data were analysed by Anova (SAS) using a sire within line model after the Log_{10}-transformation was applied to normalise distributions.

RESULTS

Mean FEC for adult ewes in R lines were significantly lower than those in the S line on all occasions except May 15 prior to lambing (Table 1). Expected lambing cycle and pregnancy status (empty or pregnant) did not have an effect on FEC except for one sampling, 6 weeks after lambing commenced. Average FEC before lambing were 18, 8 and 150 epg for the LR, ER and S lines, respectively.

TABLE 1 Seasonal least squares geometric mean (Log_{10}(FEC+1)) nematode faecal egg counts (epg) for Perendale ewes after mating in 1990

Sample Date	Selection Line: Late Resistant	Early Resistant	Susceptible	Probability
May 15	1.25(0.37)[a]	0.99(0.31)	1.60(0.32)	NS
Jun 06	1.17(0.33)	0.96(0.33)	1.78(0.30)	*
Jun 12	0.89(0.28)	0.67(0.26)	2.25(0.25)	***
Jun 20	0.98(0.30)	0.67(0.28)	2.09(0.28)	***
Jun 26	0.50(0.31)	0.29(0.29)	1.48(0.29)	***
Jul 03	1.37(0.31)	1.05(0.29)	2.13(0.30)	**
Jul 11	1.82(0.28)	1.53(0.27)	2.60(0.27)	***
Jul 19	1.63(0.27)	1.19(0.26)	2.61(0.25)	***
Jul 30	2.03(0.29)	1.67(0.27)	2.80(0.27)	***
Aug 13	1.24(0.31)	0.60(0.31)	2.50(0.33)	***
Sep 26	2.60(0.25)	2.03(0.26)	2.79(0.27)	NS
Oct 19	2.15(0.30)	2.33(0.29)	2.88(0.28)	NS
Nov 13	0.85(0.32)	0.74(0.31)	1.68(0.32)	*
Nov 27	1.57(0.62)	0.83(0.38)	2.38(0.53)	NS

[a] Mean (S.E.); *** = P<0.001; ** = P<0.01; * = P<0.05; NS = Not Significant

A periparturient rise of FEC occurred and peaked approximately 3-6 weeks after lambing commenced. FEC of resistant ewes were lower on all sample dates but they were significantly lower than those of the susceptible ewes 2 weeks prior to and 9 weeks after lambing (Table 2). On average the FEC of ER ewes during late pregnancy and early lactation were about one tenth of the FEC of S ewes.

TABLE 2 Geometric least squares mean (Log_{10}(FEC+1)) faecal egg counts (eggs/g) for pregnant/lactating Perendale ewes during the periparturient period

Selection Line	Time to Lambing (weeks): -2	3	6	9
Late Resistant	1.47	2.33	1.57	1.60
Early Resistant	1.22	2.45	1.62	1.10
Susceptible	2.68	2.99	2.33	2.36
Effects				
Line	***	NS	NS	**
Lambing Cycle	**	NS	*	NS
Year of birth	NS	NS	NS	NS
Line x Cycle	NS	NS	NS	NS

*** = P<0.001; ** = P<0.01; * = P<0.05; NS = Not Significant

Sex of lamb did not have a significant effect on FEC throughout the study (Table 3). While grazing together, FEC of ram lambs were not consistently higher then those of the ewes. Consistently, FEC was higher in the S lambs than either LR or ER lambs. On 30 April 6- and 8-fold differences in back-transformed means were recorded. Faecal cultures demonstrated that *Haemonchus* and *Trichostrongylus* were the dominant genera (Table 4).

TABLE 3 Geometric least squares mean (Log_{10}(FEC+1)) summaries for nematode faecal egg counts (epg) for Perendale progeny born in 1990.

Line[a]	Sample Date: Weaning	14 Jan	18 Feb	10 Apr	30 Apr
LR	2.11	3.17	2.54	2.27	2.65
ER	2.24	3.11	2.42	2.20	2.50
S	2.68	3.61	2.70	3.14	3.42
Effects					
Sex	NS	NS	NS	NS	NS
Sire within line	NS	**	NS	*	*
Line	NS	***	NS	***	***

[a] LR = Late (acquired) resistant; ER = Early (Immune) resistant; S = Susceptible; *** = P<0.001; ** = P<0.01; * = P<0.05; NS = Not Significant

TABLE 4 Faecal larval cultures for Perendale hoggets (born 1990).

Selection Line	Date	Nematode Species (%): Coop	Haem	Trich	Other
Late Resistant	21/2/91	2	84	10	4
Early Resistant	-	18	52	24	6
Susceptible	-	2	62	22	14
Late Resistant	11/4/91	30	38	24	8
Early Resistant	-	6	56	26	12
Susceptible	-	4	68	26	2
Late Resistant	30/4/91	14	18	50	18
Early Resistant	-	4	18	66	12
Susceptible	-	8	50	36	6

Coop = *Cooperia*; Haem = *Haemonchus*; Trich = *Trichostrongylus*
Other = *Ostertagia, Nematodirus, Chabertia, Oesophagostomum*

Least squares means for weaning weight, GGT, fleece weight and liveweight gains (Gain) for the progeny are summarised in Table 5. Sire had a significant effect on Gain and FWT. GGT level and WWT were significantly affected by selection line.

TABLE 5 Least squares geometric performance trait means ($Log_{10}(X)$) for Perendale progeny born in 1990.

Line	WWT (kg)	Gain (kg)	FWT (kg)	GGT (iu/l)
LR	1.26	1.10	2.99	1.53
ER	1.29	1.10	3.03	1.53
S	1.30	1.12	3.05	1.56
Effects				
Sex	NS	NS	NS	NS
Sire within line	NS	***	*	NS
Line	*	NS	NS	*

WWT = weaning weight; Gain = April Weight - WWT; FWT = Fleece weight; GGT = gamma glutamyl transferase; LR = late resistant; ER = early resistant; S = susceptible; *** = P<0.001; ** = P<0.01; * = P<0.05; NS = Not Significant

DISCUSSION

After approximately 5 years of selective mating of Perendale sheep for increased or decreased FEC following experimental infection with *H. contortus* genetic variation has been demonstrated. As a consequence 3 lines have been now established, 2 for low and another for high FEC.

Ewes selected as lambs for ER or LR consistently exhibited lower FEC than their S counterparts during the 7 months they were compared after experimental infection with *H. contortus*. This pattern for FEC spanned across pregnancy and parturition.

Similar responses have been well documented elsewhere with other sheep breeds. Australian workers have investigated changes in selected Merino flocks after challenge with *H. contortus* or *T. colubriformis*. In long term studies, breeding ewes selected for resistance had lower FEC than unselected animals up to 5 years after the experimental challenge (see Gray, 1991).

Studies involving relatively unimproved sheep have shown that changes in FEC associated with reproductive cycle have distinct breed variations. Preston and Allonby (1979) found seasonality extended across breeds when they demonstrated that Red Maasai sheep had lower FEC than Merino, Corriedale, Hampshire Down, Dorper and Black Persian breeds over most of a 2-year period during which they were monitored.

There is a large body of evidence that ewe immunity to infection by nematode parasites tends to relax prior to lambing. The phenomenon is commonly referred to as the peri-parturient rise (PPR). More recently, various researchers have demonstrated that there is some genetic basis to this decline in responsiveness. Courtney *et al.*, (1984) showed that Florida Native ewes had significantly lower nematode burdens than cross-bred domestic ewes and did not exhibit a PPR. Subsequently, Zajac *et al.*, (1988) found that Florida Native ewes exhibited significantly lower FEC than Rambouillet across pregnancy and the expected PPR period after confinement.

PPR was not suppressed in the R line ewes however, FEC were lower than those of S ewes on all occasions and R ewes appeared to respond rapidly to eliminate infections effectively. Two weeks prior to lambing R lines had FEC approximately 50% lower than that of the S line. FEC peaked 3 weeks after lambing in all lines however, ER and LR lines had approximately 20% lower FEC. Thereafter, FEC of R lines were 30 to 50% lower than those of the S line. These data suggest that selection differential between the R and S lines will remain despite the reproductive status of the ewe. As a consequence, R ewes will assist to reduce seasonal pasture contamination during lambing by developing a lower PPR peak as well as possibly responding to the re-infection more effectively.

Lambs from the various lines maintained respective differences from weaning until they were approximately 8 months of age when monitoring was discontinued. In fact, there was over a 10-fold difference between progeny from the most extreme sires when the study was terminated. Under normal practice based on FEC, LR or ER lambs would not have been given parasite treatment after 18 February whereas S lambs would have required treatment on or before 10 April given that the predominant species were *Haemonchus* and *Trichostrongylus*.

This pattern for lambs in selection flocks is not restricted to Perendale sheep. Woolaston, Barger and Piper (1990) observed that Merino lambs in a flock selected following experimental infection with *H. contortus* retained their responsiveness between weaning and 12 months of age.

The present experiment has demonstrated that there are genetic variations for FEC in Perendale sheep and that these may be used for breeding purposes. The fact that there is a genetic basis to the FEC in Perendales is not surprising given the data generated in genetic selection and comparative studies conducted to date with other breeds. Heritabilities estimated on 34 sires used prior to 1990 to develop the Perendale lines used in this study ranged between 0.12 and 0.57 depending on the FEC used (Watson, Unpubl.). These are similar to those calculated for Romney sheep (0.29. -0.39) following natural mixed infection (Watson *et al.*, 1986; Baker *et al.*, 1991; Bisset *et al.*, 1991; McEwan *et al.*, 1992) and reported for Merinos (0.22-0.33) following experimental infection (Woolaston *et al.*, 1991).

On the basis of the present data, ewes and their lambs selected for high or low FEC will tend to display FEC consistent with their selection. This suggests that resistant ewes will help to minimise contamination of pasture during lambing and may, in fact, assist to clean up the environment.

Challenge with restricted parasite species may provide opportunities to increase selection pressure directly towards specific nematode species. This will only be assessed in time. In the meantime, two important observations have arisen from this experiment. Firstly, selection following artificial challenge with *H. contortus* has not hindered animals responding to the mixed infections they would be expected to encounter while grazing. Secondly, animals continue to remain resistant to infection (based on FEC) into their adult years.

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