

**Final Report to the Ministry of Agriculture and Forests - Sustainable Farming Fund**

**Investigation of Possible Links between  
a Sheep's Ability to Combat Footrot  
and to Resist Internal Parasites  
based on the Footrot Gene Marker Test**

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# Investigation of Possible Links between a Sheep's Ability to Combat Footrot and to Resist Internal Parasites based on the Footrot Gene Marker Test

## Executive Summary

Investigations on five breeding properties revealed that drench resistance was emerging on all the properties to white drenches (BZ drenches) and on two properties to Ivomec drenches (ML drenches). There was no evidence of resistance to clear drenches (LV drenches) on the five properties in this investigation. On some of the properties the effectiveness of specific white drenches was very low. This supports the contention that the useful lives of many drenches are nearing their end and better parasite management strategies are needed immediately.

The mix of parasites found in sheep on any of the five given properties was different and it can be concluded that there is no precise way of knowing the species present, other than to count and measure. Similar properties with the same breed of sheep had quite different parasite species present and with different levels of drench resistance. This suggests that drench resistance is emerging from multiple primary sources.

ANOVA revealed that there were significant sire-line differences in EPG 1 (weaning), EPG 2 (autumn) and weaning weight on the majority of the properties for which sire information was available. This supports the notion that breeders could select sheep that have lower faecal egg counts (FEC's) for breeding (which is already happening in the Nemesis programme in Australia) and is supported by heritability estimates from the literature for FEC ranging from 0.2 at weaning to 0.7 in older sheep.

An association was found between alleles of the Major Histocompatibility Complex gene DQA2 and FEC for *Nematodirus spp.* on the three farms for which DQA2 typing was undertaken. This is an exciting preliminary result and while it doesn't describe the role of any given allele of the DQA2 gene in regulating FEC for *Nematodirus spp.* a more comprehensive analysis with many more sheep may reveal sufficient information to allow development of a parasite resistance gene-marker.

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## Introduction

### 1) Scientific Background

There is a body of scientific literature linking genes in the sheep Major Histocompatibility Complex (MHC) and the ability of sheep to resist infection by gastrointestinal parasites as measure by Faecal Egg Count (FEC) (Table 1).

Table 1. Scientific Reports linking MHC and FEC

Investigators	Nature of MHC parasite link
Schwaiger <i>et al.</i> (1995)	- alleles of DRB1 locus affects FEC
Stear <i>et al.</i> (1996)	- class I and FEC for <i>O. circumcincta</i> .
Buitkamp <i>et al.</i> (1996)	- class I and DY loci affect FEC
Paterson <i>et al.</i> (1998)	- class I and DRB loci affect FEC for <i>O. circumcincta</i>

These findings are perhaps not surprising given the critical role of these genes in controlling specific immune responses. The “MHC effect” is however estimated to be small accounting for about 11% of total phenotypic variation, although it is a somewhat larger proportion of the additive genetic variation (Stear *et al.*, 1997) (i.e. other genes are also implicated in resistance to parasites).

The above findings have led many to speculate that the MHC contains genes that could be used as markers for breeding to reduce FEC. Flocks could then be bred, that contaminate pasture less with eggs and subsequently fewer larvae would hatch to re-infect sheep and thereby continue the parasites life cycle. Even without the use of gene markers, breeders both within New Zealand and internationally are selecting rams based on them having lower FEC’s or estimated breeding values (EBV’s) for FEC, with some success reported from Australia. In New Zealand this approach has been deemed somewhat less desirable as there is some science showing negative consequences. As noted by one research group:

*“Although genetically low FEC Romney lambs have been shown to have significantly reduced burdens of the most important nematode species, they nevertheless appear to have no significant production advantages over their higher FEC counterparts when all are grazed together under the same larval challenge. Furthermore there is evidence of an unfavourable association between FEC and dagginess (soiling of the breech area) suggesting that some forms of host response to nematode challenge may result in diarrhoea. Our results suggest that the main benefits of selectively breeding for low FEC are likely to be derived indirectly as a result of reduced pasture contamination.”*  
(Bisset and Morris, 1996).

Despite this somewhat negative description of the lack of benefit in breeding for FEC, more recent research has revealed a positive correlation between resistance to parasites and body size in lambs (Coltmann *et al.* 2001), the difference possibly being that in Bisset and Morris’s work the low and high FEC sheep were grazed together, such that the benefit accrued from having lower levels of pasture contamination from some sheep were not realised.

### 2) The Footrot Gene Marker Technology

In 2001 Lincoln University introduced a gene technology that enabled sheep breeders to select sheep that were less susceptible to the bacterial hoof infection footrot. This technology involves the typing of a sheep MHC gene called DQA2, and different alleles of this gene are associated with sheep having increased or decreased susceptibility to the disease. This gene marker technology was

rapidly taken up by New Zealand fine wool and mid-micron sheep breeders in their quest to limit the costly impact of footrot and this was done with the assistance of the Ministry of Agriculture and Forests - Sustainable Farming Fund. Descriptions of the technology, its uptake and impact are available from the authors of this report.

### **3) Anecdotal Evidence that Sheep that are Selected for Footrot Tolerance have lower FEC's or are More Resilience to Parasites**

As the footrot gene marker technology became established a number of the sheep breeders using the gene marker technology reported that the sheep selected to be less susceptible to footrot appeared to be either more resistant to parasites (i.e. had lower FEC's) or were more resilient to parasites (grew at a faster rate).

Specifically, Mr Gordon Levet of Wellsford, who has been breeding Romney sheep based on phenotypic selection for low FEC and sound hooves for at least 20 years reported publicly (Country-wide Southern December 2002), then at a meeting at his farm in 2003 and then again publicly (Straight Furrow 15/9/2004) that his best rams for FEC also had the best possible scores for tolerance to footrot with the footrot gene marker technology. To him this suggested a genetic link between the two traits.

We suggested to Mr Levet at the time that this may merely be the consequence of co-selection for two independent (but valuable) traits, although it raised the possibility immediately that the MHC gene scored in the footrot gene test (DQA2) was also important (as suggested by the published science) in controlling FEC.

This speculation led to the development of a proposal for the Ministry of Agriculture and Forests Sustainable Farming Fund to shoulder on the existing programme that was underwriting the introduction of the footrot gene marker technology and investigate formally whether a DQA2 versus FEC link existed. Subsequently FEC's were measured for lambs on a select group of properties already using the footrot technology and these FEC's were compared with the DQA2 alleles that these sheep possess as described below.

## Project Description

It was proposed that an investigation study be undertaken from 1/10/2003 to the 1/6/2004 to look at the possible links between tolerance to footrot (based on the footrot gene-marker test) and worm burdens of newly weaned ram lambs as measured by FEC.

This initial project was subsequently extended to look at drench resistance and a second FEC test was done on all ram lambs in the autumn of 2004 (approximately 7-8 months of age).

A total of five large stud flocks were selected for the investigation, these consisted of two merino studs, two Corriedale studs and one Polwarth stud (Table 2).

Farmer	Locality	Sample number	Approximate date of sampling (@ weaning)
Alistair Campbell	Alexandra	515 ram lambs	Mid-February
Martin Paterson	Alexandra	339 ram lambs	Second week January
Robyn Wilson/ Stephen Field	Christchurch	342 ram lambs	Early December
Ian Stevenson	Cheviot	142 ram lambs	Early January
John Sidey	Waipara	520 ram lambs	Early December
	TOTAL	1858 ram lambs	

Given that approximately 50% of these ram lambs would have been gene tested for the footrot gene-marker as a matter of course under the existing MAF-SFF footrot program the project was planned so as to cover the additional cost that the farmers would incur. This meant that the project covered the full cost of testing 50% of the ram lambs and assumed that Lincoln University continued to provide access to the low price for gene testing made available to the MAF-SFF footrot project.

## Method

### 1) Faecal Egg Counting

Faecal samples were collected by digital extraction from ram lambs only on all properties. Rectal samples were collected on-farm by standardised protocols and processed to 30epg sensitivity using a 1 mL aliquot for examination and counting. Eggs were identified as either *Strongyle* (STRON) or *Nematodirus* (NEM). FEC results were matched to brass ID tags and sent to Lincoln University

### 2) Blood collection for DNA testing

Blood from each ram lamb was collected onto FTA cards using electrical side-cutters to clip nicks in the lambs' ears.

### 3) Drench Reduction Tests

On each property small groups of ram lambs were selected for drench reduction tests (See Appendix 1). Following the initial faecal collection for FEC determination, these lambs were drenched with the farmer's drenches of choice according to the suppliers recommended instructions and lamb bodyweights. Faecal samples were subsequently collected 10 days later for re-counting.

### 4) Parasite Species Identification

On each property pooled faecal samples were collected and sent to Dr Paul Mason for parasite species identification.

### 5) DNA Typing

DNA typing was carried out by the standard typing method of the footrot typing laboratory at Lincoln University. This is as follows:

#### *DNA Extraction*

DNA was extracted from blood collected on FTA Classic cards (*Whatman*<sup>®</sup> BioScience, Newton, MA), following the manufacturers protocol.

#### *PCR Amplification*

Two PCR primers for amplifying the entire second exon (249 bp), flanked by 209 bp of intron 1 and 370 bp of intron 2 sequences of the ovine *DQA2* gene, were designed based on two published ovine *DQA2* sequences (M33305, Scott et al., 1991; Z28421, Wright and Ballingall, 1994) with comparison to a published *DQA1* sequence (M33304, Scott et al., 1991). These primers were *DQA2*-up (5'-CACATGTTACAGTGCAAAARCAGC-3', where R means A or G) and *DQA2*-dn (5'-CCCTCYCACCAACGTTTCCCAG-3', where Y means C or T). Subsequently, internal PCR primers *DQA2s*-up (5'-ACTACCAATCTCATGGTCCCTCT-3') and *DQA2s*-dn (5'-GGAGTAGAATGGTGGACACTTACC-3') were designed to amplify the variable region of exon 2 for SSCP analysis based on the sequence information obtained in this study. Primers were synthesized by Proligo (Proligo LLC, Colorado, CA).

Amplifications for allele detection used a non-proof reading *Taq* DNA polymerase (Qiagen, Hilden, Germany), while amplifications for allele cloning and sequencing used the proof reading enzyme, ProofStart<sup>™</sup> DNA polymerase (Qiagen), in order to reduce PCR-associated nucleotide substitutions. Each PCR was performed in a 20 µL reaction volume containing 50 ng genomic DNA from whole blood or genomic DNA on one 1.2 mm punch of an FTA card, 0.25 µM of each primer, 150 µM of dNTP's (ABgene, Surrey, United Kingdom), 1 U DNA polymerase and 1× reaction buffer supplied (containing 1.5 mM MgCl<sub>2</sub>). Amplification was carried out in an iCycler (Bio-Rad, Hercules, CA) and consisted of denaturation at 94°C for 2 min, followed by 32 cycles of 94°C for 30 s, 59°C (for *DQA2*-up and *DQA2*-dn) or 62°C (for *DQA2s*-up and *DQA2s*-dn) for 30 s and 72°C for 50 s (for *DQA2*-up and *DQA2*-dn) or 30 s (for *DQA2s*-up and *DQA2s*-dn). This was followed by

a final extension step at 72°C for 5 min. Amplimers were visualized by electrophoresis in 1% Seakem<sup>®</sup> LE agarose (BioWhittaker Molecular Applications, Rockland, ME) gels using 1× TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na<sub>2</sub>EDTA), containing 200 ng/mL ethidium bromide.

#### *Single-Strand Conformational Polymorphism Analysis*

A 0.7 µL aliquot of each amplimer was mixed with 7 µL of loading dye (98% formamide, 10 mM EDTA, 0.025% bromophenol blue, 0.025% xylene-cyanol). After denaturation at 95°C for 5 min, samples were cooled rapidly on wet ice and then loaded on 16 cm × 18 cm, 14% acrylamide:bisacrylamide (37.5:1) (Bio-Rad) gels. Electrophoresis was performed using Protean II xi cells (Bio-Rad), at 380 V for 18 h at 5 °C in 0.5× TBE buffer. Gels were silver-stained according to the method of Bassam et al. (1991).

Alleles were described by their common letter names and not the scientific names described in Hickford *et al.* 2004. These common names translate to the ISAG and GenBank nomenclature as follows:

#### Ovine *DQA2* alleles

Formal name	Common name	GenBank accession no.	Note
0101	Ga	AY312375	
0102	Fa	AY312376	
0103	D	AY312377	
0201	Ib	AY312378	<i>DQA2</i> -like
0301	K	AY312379	
0401	J1a	AY312396	
0402	Sa	AY312397	
0501	L	AY312380	
0601	B2	AY312381	
0602	B1	AY312382	
0701	Ja	AY312383	
0702	J2a	AY312384	
08011	C1	AY312385	
08012	Ia	AY312386	
0901	C2	AY312387	
1001	Q	AY312388	
1101	E	AY312389	
1201	H	AY312390	
1301	J'b	AY312391	<i>DQA2</i> -like
1401	Gb	AY312392	<i>DQA2</i> -like
1501	J1b	AY312393	<i>DQA2</i> -like
1601	Fb	AY312394	<i>DQA2</i> -like
1701	Sb	AY312395	<i>DQA2</i> -like

## 6) Statistical Analysis

Results data was entered into spreadsheets and then analysed using SPSS.

## Project Management

Time was input by Jon Hickford and John Bates to managing the project.

Jon Hickford managed the operation of the footrot gene-typing laboratory and blood collection for DNA-typing. He co-ordinated the collation of data, the scientific analysis and reporting to the farmers and funders.

John Bates managed the project funds received from MerinoNZ Inc., Mid-micron Wools of NZ Inc. and MAF-SFF. He co-ordinated the FEC and data collections.

FECPAK was contracted to develop the testing protocols and physically take the faecal samples.

## Results

### 1) Drench Reduction Test Results

Drench reduction test results are listed in **Appendix 1**.

Four out of the five properties tested showed some degree of parasite drench resistance to white drench.

Results varied from property to property.

Typically parasite resistance to white drenches was most commonly seen. The average reduction (i.e across the group of lambs), varied from 58.7% on one property to 96.56% on another.

Four of the five properties showed an “across species” resistance to both *Nematodirus* (NEM) and *Strongyle* (STRON) parasites, while the property that had a 96.56% level, appeared to have drench resistance developing in NEM only and for this parasite the reduction rate was 92%. Two other properties had a reduction rate for NEM of only 28% and 43%. For these properties it would seem that NEM is much more resistant to white drench than the STRON species and that the level of drench resistance can be very high for NEM.

All properties showed no parasite resistance to clear drenches

Both a half Ivomec and full Ivomec drench were tested. Two properties showed parasite resistance to a full Ivomec drench (reduction of 82% and 83%). Only STRON species showed drench resistance on those properties.

### 2) Parasite Species Present

The parasite species (**Appendix 2**) varied greatly between properties at the different times of measurement. It was interesting that properties 3 and 5 have the same breed of sheep (Corriedale) in very similar locations and yet have quite different parasite species present. Property 3 in particular, had a very high summer level of *Trichostrongylus* at a time when most properties were *Ostertagia* dominated.

### 3) Sire-line Differences in FEC

Analyses (**Appendix 3**) were done on the three properties where we had confidence in the sire information. Sirelines with less than five progeny for which data were recorded were discarded and individual lambs with missing data were either discarded or used if the data recorded could be analysed.

#### *Correlation Analysis*

In the first analysis correlation coefficients were calculated between the various traits measured. Firstly, EPG 1 (weaning) was correlated with EPG 2 (autumn) within sire lines (**Appendix 3A**). These coefficients ranged from -0.377 to 0.876, with mean across sire-line coefficients on the three farms of 0.156 (Patterson), 0.357 (Wilson) and 0.101 (Campbell).

Comparison of EPG 1 (Weaning) and weaning weight WWT gave a correlation coefficient of 0.238 for Wilson and -0.138 for Campbell. When EPG 1 was split to NEM and STRON, the correlation coefficients were 0.178 (STRON) and 0.227 (NEM) for Wilson and -0.096 (STRON) and -0.104 (NEM) for Campbell.

#### *Sire Effect Analysis*

One way ANOVA's (**Appendix 3B**) were undertaken to analyse the effect of sire-line on the individual traits measured. These analyses revealed a significant relationship between sire-line and WWT (n= 230, P<0.000) and a trend suggesting a relationship between sire-line and EPG 1 (n= 251, P=0.051) for Wilson.

With Campbell there were significant relationships between EPG 1 (n= 502, P=0.025), EPG 2 (n=471, P=0.017), WWT (n=505, P<0.000) and sire-line.

With Patterson lambs were farmed separately on two properties. On property one (R), there were no significant effects of sire on EPG 1 (n=147), EPG 2 (n=148) or WWT (n= 135), while on property two (M), EPG 1 (n=181, P=0.029) was significantly associated with sire-line while EPG 2 (n=181) and WWT (n=165) were not. Overall for Patterson, when the data from both properties was combined, only EPG 1 (n=329 P<0.002) was significantly associated with sire-line.

#### *Within Sire-line Gene Analysis*

T-tests (**Appendix 3C**) were performed on the segregation of individual DQA2 alleles and variation in the measured traits (EPG 1, EPG 1, NEM 1, NEM 2, STRON 1, STRON 2, WWT) for all sire-lines where the sire was heterozygous at DQA2 and there were more than 12 progeny. Progeny that typed the same as the sire were excluded from the analysis.

Once again these analyses were undertaken for the three farms described above. Results are summarised below and in the following tables.

There was a significant within-sire differences in mean NEM 1 for allele D (mean<sub>NEM 1</sub> = 2.6) and allele H (mean<sub>NEM 1</sub> = 7.9) for sire Patterson RAY (P = 0.004). This can probably also explain the significant within-sire difference in mean EPG 1 for allele D (mean<sub>EPG 1</sub> = 330.0) and allele H (mean<sub>EPG 1</sub> = 945.0) for this sire (P = 0.024). There was also a significant within-sire differences in mean NEM 1 for allele C2 (mean<sub>NEM 1</sub> = 9.6) and allele J1 (mean<sub>NEM 1</sub> = 4.6) for sire Patterson RAY (P = 0.045).

There was a significant within-sire differences in mean EPG 2 for allele F2 (mean<sub>EPG 2</sub> = 1172.7) and allele H (mean<sub>EPG 2</sub> = 1725.0) for sire Campbell S959 (P = 0.049).

There was a significant within-sire differences in weaning weight for allele C2 (mean<sub>WWT</sub> = 28.2) and allele G (mean<sub>WWT</sub> = 33.1) for sire Wilson WF606-99 (P = 0.031).

#### *Within Farm Gene Analysis*

General Linear Models (GLM) (**Appendix 3D**) were created for a univariate analysis of variance of genotype at DQA2 versus the measured traits (EPG 1, EPG 1, NEM 1, NEM 2, STRON 1, STRON 2, WWT) across all sire-lines on any given property, correcting for variation between the sires.

Results are summarised below and in the following table.

On the Wilson property there was a significant effect of DQA2 genotype on EPG 2 (P=0.043), STRON 2 (P=0.004) and NEM 1 (P=0.007). On the Campbell property there was a significant effect of DQA2 genotype on NEM 1 (P<0.000) and on the Patterson property there was a significant effect of DQA2 genotype on EPG 1 (P=0.019), EPG 2 (P=0.050) and NEM 1 (P=0.015).

**Table 1. Segregation of MHC DQA2 alleles and EPG 1**

Sire and Farm	Sire Genotype (No. of progeny with that allele bracketed)	Mean EPG 1 (Allele 1 bracketed)	Mean EPG 1 (Allele 2 bracketed)	Significance
Campbell BWG926	C2 (11), J1 (14)	640.9 (C2)	608.6 (J2)	P=0.817
Campbell KT113	G (5), K(10)	834.0 (G)	741.0 (K)	P=0.635
Campbell N49	B1 (12), J2 (16)	566.3 (B1)	727.5 (J2)	P=0.205
Campbell S955	E (19), H (23)	750.0 (E)	652.2 (H)	P=0.429
Campbell S959	F2 (13), H (21)	830.8 (F2)	611.4 (H)	P=0.119
Campbell T222	E (17), J1 (26)	790.6 (E)	776.5 (J1)	P=0.916
Campbell T283	H (4), L (12)	1020.0 (H)	737.5 (L)	P=0.164
Campbell T421	G (26), L(21)	766.2 (G)	810.0 (L)	P=0.758
Campbell T429	C2 (6), J1(7)	1185.0 (C2)	942.9 (J1)	P=0.107
Campbell T432	E (7), J2 (7)	745.7 (E)	711.4 (J2)	P=0.884
Campbell T435	E (13), G (7)	904.6 (E)	1461.4 (G)	P=0.262
Campbell T440	E (7), H (12)	831.4 (E)	672.5 (H)	P=0.428
Wilson E136-00	B1 (14), G (16)	726.4 (B1)	579.4 (G)	P=0.532
Wilson WF597-99	B1 (18),G (21)	658.3 (B1)	698.6 (G)	P=0.835
Wilson WF598-99	F2 (9), G (20)	823.3 (F2)	528.0 (G)	P=0.415
Wilson WF606-99	C2 (8), G (14)	435.0 (C2)	233.6 (G)	P=0.160
Wilson WF 620-01	C2 (10), G (7)	861.0 (C2)	501.4 (G)	P=0.391
Patterson EKO2	H (17), S (5)	460.6 (H)	618.0 (S)	P=0.447
Patterson KEV	B1 (10), H (16)	735.0 (B1)	1128.8 (H)	P=0.087
Patterson NIK	H (14), J1 (17)	801.4 (H)	961.8 (J1)	P=0.398
<b>Patterson RAY</b>	<b>D (7), H (10)</b>	<b>330.0 (D)</b>	<b>945.0 (H)</b>	<b>P=0.024</b>
Patterson ROM	G (10), H (17)	609.0 (G)	794.1 (H)	P=0.408

**Table 2. Segregation of MHC DQA2 alleles and EPG 2**

Sire and Farm	Sire Genotype (No. of progeny with that allele bracketed)	Mean EPG 2 (Allele 1 bracketed)	Mean EPG 2 (Allele 2 bracketed)	Significance
Campbell BW926	C2 (11), J1 (13)	2462.7 (C2)	2695.4 (J2)	P=0.726
Campbell KT113	G (5), K(10)	1278.0 (G)	1584.0 (K)	P=0.524
Campbell N49	B1 (13), J2 (16)	1933.1 (B1)	2319.2 (J2)	P=0.400
Campbell S955	E (14),H (21)	2112.1 (E)	2041.4 (H)	P=0.860
<b>Campbell S959</b>	<b>F2 (11), H (20)</b>	<b>1172.7 (F2)</b>	<b>1725.0 (H)</b>	<b>P=0.049</b>
Campbell T222	E (14), J1 (21)	2206.0 (E)	2750.9 (J1)	P=0.365
Campbell T283	H (4), L (12)	1702.5 (H)	1887.5 (L)	P=0.767
Campbell T421	G (25), L(18)	1436.4 (G)	1993.3 (L)	P=0.160
Campbell T429	C2 (8), J1(7)	1743.8 (C2)	1881.4 (J1)	P=0.797
Campbell T432	E (7), J2 (7)	2481.3 (E)	2382.9 (J2)	P=0.900
Campbell T435	E (11), G (6)	2645.5 (E)	1780.0 (G)	P=0.357
Campbell T440	E (7), H (12)	3218.57(E)	1987.5(H)	P=0.473
Wilson E136-00	B1 (7), G (5)	312.9 (B1)	174.0 (G)	P=0.516
Wilson WF597-99	B1 (12) ,G (17)	237.5 (B1)	240.0 (G)	P=0.973
Wilson WF598-99	F2 (5), G (5)	84.0 (F2)	183.8 (G)	P=0.298
Wilson WF606-99	C2 (6), G (7)	190.0 (C2)	201.4 (G)	P=0.899
Wilson WF 620-01	C2 (9), G (5)	356.7 (C2)	186.0 (G)	P=0.492
Patterson EKO2	H (17), S (4)	86.5 (H)	90.0 (S)	P=0.948
Patterson KEV	B1 (9), H (14)	73.3 (B1)	100.7 (H)	P=0.602
Patterson NIK	H (14), J1 (17)	27.9 (H)	56.5 (J1)	P=0.299
Patterson RAY	D (7), H (10)	47.1 (D)	60.0 (H)	P=0.750
Patterson ROM	G (10), H (17)	57.0 (G)	60.0 (H)	P=0.887

**Table 3. Segregation of MHC DQA2 alleles and WWT**

Sire and Farm	Sire Genotype (No. of progeny with that allele bracketed)	Mean WWT (Allele 1 bracketed)	Mean WWT (Allele 2 bracketed)	Significance
Campbell BW926	C2 (11), J1 (14)	27.5 (C2)	25.9 (J2)	P=0.338
Campbell KT113	G (5), K(10)	29.4 (G)	29.9 (K)	P=0.838
Campbell N49	B1 (16), J2 (12)	25.1 (B1)	27.2 (J2)	P=0.267
Campbell S955	E (19), H (23)	28.7 (E)	29.6 (H)	P=0.574
Campbell S959	F2 (13), H (21)	29.2 (F2)	29.4 (H)	P=0.896
Campbell T222	E (18), J1 (25)	29.4 (E)	30.3 (J1)	P=0.612
Campbell T283	H (4), L (12)	27.2 (H)	27.1 (L)	P=0.940
Campbell T421	G (26), L(21)	26.9 (G)	27.8 (L)	P=0.481
Campbell T429	C2 (7), J1(6)	28.3 (C2)	27.8 (J1)	P=0.809
Campbell T432	E (7), J2 (7)	24.7 (E)	27.0 (J2)	P=0.345
Campbell T435	E (13), G (7)	26.9 (E)	26.6 (G)	P=0.868
Campbell T440	E (7), H (13)	25.7 (E)	26.8 (H)	P=0.625
Wilson E136-00	B1 (13), G (16)	36.9 (B1)	38.2 (G)	P=0.584
Wilson WF597-99	B1 (18),G (21)	34.0 (B1)	35.8 (G)	P=0.348
Wilson WF598-99	F2 (9), G (20)	32.1 (F2)	32.1 (G)	P=0.988
<b>Wilson WF606-99</b>	<b>C2 (8), G (14)</b>	<b>28.2 (C2)</b>	<b>33.1 (G)</b>	<b>P=0.031</b>
Wilson WF 620-01	C2 (10), G (7)	35.2 (C2)	34.1 (G)	P=0.654

**Table 4. Segregation of MHC DQA2 alleles and NEM 1**

Sire and Farm	Sire Genotype (No. of progeny with that allele bracketed)	Mean NEM 1 (Allele 1 bracketed)	Mean NEM 1 (Allele 2 bracketed)	Significance
<b>Campbell BW926</b>	<b>C2 (11), J1 (14)</b>	<b>9.6 (C2)</b>	<b>4.6 (J2)</b>	<b>P=0.045</b>
Campbell KT113	G (5), K(10)	7.0 (G)	8.3 (K)	P=0.732
Campbell N49	B1 (16), J2 (12)	5.9 (B1)	8.8 (J2)	P=0.230
Campbell S955	E (19), H (23)	9.2 (E)	7.4 (H)	P=0.337
Campbell S959	F2 (13), H (21)	7.5 (F2)	6.8 (H)	P=0.555
Campbell T222	E (17), J1 (26)	6.9 (E)	9.1 (J1)	P=0.206
Campbell T283	H (4), L (12)	12.5 (H)	9.2 (L)	P=0.482
Campbell T421	G (26), L(21)	9.5 (G)	11.2 (L)	P=0.537
Campbell T429	C2 (6), J1(7)	22.5 (C2)	17.9 (J1)	P=0.457
Campbell T432	E (7), J2 (7)	18.3 (E)	17.3 (J2)	P=0.876
Campbell T435	E (13), G (7)	18.5 (E)	35.0 (G)	P=0.247
Campbell T440	E (7), H (12)	16.3 (E)	11.6 (H)	P=0.352
Wilson E136-00	B1 (14), G (16)	9.4 (B1)	6.8 (G)	P=0.459
Wilson WF597-99	B1 (18),G (21)	9.4 (B1)	9.6 (G)	P=0.968
Wilson WF598-99	F2 (9), G (20)	11.0 (F2)	9.7 (G)	P=0.834
Wilson WF606-99	C2 (8), G (14)	3.3 (C2)	3.7 (G)	P=0.743
Wilson WF 620-01	C2 (10), G (7)	14.6 (C2)	7.4 (G)	P=0.506
Patterson EKO2	H (17), S (5)	5.2 (H)	5.8 (S)	P=0.880
Patterson KEV	B1 (10), H (16)	6.6 (B1)	11.6 (H)	P=0.130
Patterson NIK	H (14), J1 (17)	8.9 (H)	10.8 (J1)	P=0.525
<b>Patterson RAY</b>	<b>D (7), H (10)</b>	<b>2.6 (D)</b>	<b>7.9 (H)</b>	<b>P=0.004</b>
Patterson ROM	G (10), H (17)	10.5 (G)	9.7 (H)	P=0.783

**Table 5. Segregation of MHC DQA2 alleles and STRONG 1**

<b>Sire and Farm</b>	<b>Sire Genotype (No. of progeny with that allele bracketed)</b>	<b>Mean STRONG 1 (Allele 1 bracketed)</b>	<b>Mean Strong 2 (Allele 2 bracketed)</b>	<b>Significance</b>
Campbell BW926	C2 (11), J1 (14)	11.7 (C2)	15.6 (J1)	P=0.268
Campbell KT113	G (5), K(10)	20.8 (G)	16.4 (K)	P=0.383
Campbell N49	B1 (16), J2 (12)	12.9 (B1)	15.4 (J2)	P=0.551
Campbell S955	E (19), H (23)	15.8 (E)	14.4 (H)	P=0.618
Campbell S959	F2 (13), H (21)	20.2 (F2)	13.6 (H)	P=0.109
Campbell T222	E (17), J1 (26)	19.5 (E)	16.8 (J1)	P=0.436
Campbell T283	H (4), L (12)	21.5 (H)	15.4 (L)	P=0.204
Campbell T421	G (26), L(21)	16.1 (G)	15.8 (L)	P=0.933
Campbell T429	C2 (6), J1(7)	17.0 (C2)	13.6 (J1)	P=0.544
Campbell T432	E (7), J2 (7)	6.6 (E)	6.4 (J2)	P=0.958
Campbell T435	E (13), G (7)	11.6 (E)	13.7 (G)	P=0.690
Campbell T440	E (7), H (12)	11.4 (E)	10.8 (H)	P=0.886
Wilson E136-00	B1 (14), G (16)	14.9 (B1)	12.5 (G)	P=0.665
Wilson WF597-99	B1 (18),G (21)	12.6 (B1)	13.7 (G)	P=0.745
Wilson WF598-99	F2 (9), G (20)	16.4 (F2)	8.0 (G)	P=0.282
Wilson WF606-99	C2 (8), G (14)	11.3 (C2)	4.1 (G)	P=0.103
Wilson WF 620-01	C2 (10), G (7)	14.1 (C2)	9.3 (G)	P=0.489
Patterson EKO2	H (17), S (5)	10.1 (H)	14.8 (S)	P=0.418
Patterson KEV	B1 (10), H (16)	17.9 (B1)	26 (H)	P=0.196
Patterson NIK	H (14), J1 (17)	17.9 (H)	21.2 (J1)	P=0.448
Patterson RAY	D (7), H (10)	8.4 (D)	23.6 (H)	P=0.079
Patterson ROM	G (10), H (17)	9.8 (G)	16.8 (H)	P=0.242

**Table 6. Segregation of MHC DQA2 alleles and NEM 2**

<b>Sire and Farm</b>	<b>Sire Genotype (No. of progeny with that allele bracketed)</b>	<b>Mean NEM 2 (Allele 1 bracketed)</b>	<b>Mean NEM 2 (Allele 2 bracketed)</b>	<b>Significance</b>
Campbell BW926	C2 (11), J1 (13)	5.7 (C2)	6.9 (J2)	P=0.746
Campbell KT113	G (5), K(10)	4.2 (G)	5.3 (K)	P=0.696
Campbell N49	B1 (13), J2 (16)	5.7 (B1)	3.5 (J2)	P=0.239
Campbell S955	E (13), H (21)	6.9 (E)	6.0 (H)	P=0.747
Campbell S959	F2 (11), H (20)	4.2 (F2)	7.7 (H)	P=0.213
Campbell T222	E (15), J1 (23)	11.1 (E)	9.8 (J1)	P=0.713
Campbell T283	H (4), L (12)	4.5 (H)	4.1 (L)	P=0.949
Campbell T421	G (25), L(18)	2.2 (G)	5.5 (L)	P=0.184
Campbell T429	C2 (8), J1(7)	7.3 (C2)	5.3 (J1)	P=0.410
Campbell T432	E (7), J2 (7)	9.3 (E)	9.9 (J2)	P=0.920
Campbell T435	E (11), G (6)	7.6 (E)	5.5 (G)	P=0.483
Campbell T440	E (7), H (12)	11.9 (E)	7.6 (H)	P=0.554
Wilson E136-00	B1 (7), G (5)	3.7 (B1)	1.4 (G)	P=0.476
Wilson WF597-99	B1 (12), G (17)	2.3 (B1)	3.1 (G)	P=0.588
Wilson WF598-99	F2 (5), G (8)	0.2 (F2)	1.5 (G)	P=0.104
Wilson WF606-99	C2 (6), G (7)	2.2 (C2)	1.0 (G)	P=0.546
Wilson WF 620-01	C2 (9), G (5)	2.6 (C2)	1.2 (G)	P=0.525
Patterson EKO2	H (17), S (4)	2.8 (H)	3.0 (S)	P=0.897
Patterson KEV	B1 (9), H (14)	2.1 (B1)	3.2 (H)	P=0.536
Patterson NIK	H (12), J1 (16)	1.0 (H)	1.9 (J1)	P=0.372
Patterson RAY	D (7), H (8)	1.6 (D)	2.4 (H)	P=0.588
Patterson ROM	G (10), H (17)	1.7 (G)	1.9 (H)	P=0.736

**Table 7. Segregation of MHC DQA2 alleles and STRONG 2**

Sire and Farm	Sire Genotype (No. of progeny with that allele bracketed)	Mean EPG 1 (Allele 1 bracketed)	Mean EPG 1 (Allele 2 bracketed)	Significance
Campbell BW926	C2 (11), J1 (13)	76.4 (C2)	82.9 (J2)	P=0.756
Campbell KT113	G (5), K (10)	38.4 (G)	47.5 (K)	P=0.542
Campbell N49	B1 (16), J2 (13)	58.8 (B1)	73.9 (J2)	P=0.301
Campbell S955	E (13), H (21)	60.8 (E)	62.1 (H)	P=0.918
Campbell S959	F2 (11), H (20)	34.9 (F2)	49.8 (H)	P=0.064
	E (15), J1 (23)	62.4 (E)	82.0 (J1)	P=0.280
Campbell T283	H (4), L (12)	52.5 (H)	58.8 (L)	P=0.768
Campbell T421	G (25), L(18)	45.6 (G)	60.9 (L)	P=0.194
Campbell T429	C2 (8), J1(7)	50.9 (C2)	57.4 (J1)	P=0.700
Campbell T432	E (7), J2 (7)	73.4 (E)	69.6 (J2)	P=0.868
Campbell T435	E (9), G (6)	80.6 (E)	53.8 (G)	P=0.389
Campbell T440	E (7), H (12)	95.4 (E)	58.7 (H)	P=0.329
Wilson E136-00	B1 (7), G (5)	6.7 (B1)	4.4 (G)	P=0.599
Wilson WF597-99	B1 (12), G (27)	5.6 (B1)	4.9 (G)	P=0.716
Wilson WF598-99	F2 (5), G (8)	2.6 (F2)	4.6 (G)	P=0.428
Wilson WF606-99	C2 (6), G (7)	4.2 (C2)	5.7 (G)	P=0.407
Wilson WF 620-01	C2 (9), G (5)	9.3 (C2)	5.0 (G)	P=0.583
Patterson EKO2	H (17), S (4)	0.1 (H)	0.0 (S)	P=0.640
Patterson KEV	B1 (9), H (14)	0.3 (B1)	0.1 (H)	P=0.301
Patterson NIK	H (12), J1 (16)	0.1 (H)	0.1 (J1)	P=0.736
Patterson RAY	D (7), H (8)	0.0 (D)	0.1 (H)	P=0.369
Patterson ROM	G (10), H (17)	0.2 (G)	0.1 (H)	P=0.141

**Table 8. Univariate GLM of DQA2 versus traits for the three properties**

Farm	Trait	Significance
Wilson	EPG 1	P=0.142
	<b>EPG 2</b>	<b>P=0.043</b>
	STRON 1	P=0.921
	<b>STRON 2</b>	<b>P=0.004</b>
	<b>NEM 1</b>	<b>P=0.007</b>
	NEM 2	P=0.866
Campbell	EPG 1	P=0.139
	EPG 2	P=0.055
	STRON 1	P=0.821
	STRON 2	P=0.059
	<b>NEM 1</b>	<b>P=0.000</b>
	NEM 2	P=0.335
Patterson	<b>EPG 1</b>	<b>P=0.019</b>
	<b>EPG 2</b>	<b>P=0.050</b>
	STRON 1	P=0.086
	STRON 2	P=0.743
	<b>NEM 1</b>	<b>P=0.015</b>
	NEM 2	P=0.077

## Discussion

### Drench Reduction Test

The drench reduction results in this study varied from property to property across the five properties studied. The results revealed that white drenches (BZ drenches) are losing their effectiveness in combating GI parasites in New Zealand. This confirms other findings both within New Zealand and internationally (see Waller 1997).

There was no evidence of incidence of clear drench (LV drench) resistance, despite other reports to this effect in New Zealand (see Waller 1997). This probably reflects the small number of properties studied. Resistance to the macrocyclic lactones (ML drench) was found on two properties confirming other similar findings both within New Zealand and Internationally Leathwick 1995, Besier 1996, Echevarria *et al.* 1996, Gopal *et al.* 2001, Mason *et al.* 1999, Leathwick *et al.* 2001. The one property using a “triple” drench (BZ, LV, and ML) showed no incidence of drench resistance.

The above findings are not extraordinary, but confirm once again the need for a better and more sustainable solution to parasite control.

### Sire-line Differences in FEC

Correlation analysis between EPG 1 (weaning) and EPG 2 (autumn) on all three properties revealed very low correlation coefficients. The relationship between EPG readings at different times is a contentious area. At the level of repeatability of FEC, there is variation from 0.22 (Pollott *et al.* 2004) to 0.46 (Morris *et al.* 1997), while Pollott *et al.* (2004) report phenotypic correlations for FEC recorded at different times over weaning (100 days) to hogget age (400 days) ranging from 0.11 to 0.54. They noted that the closer the age at which the scores were correlated the higher the correlation, but that FEC measurements at weaning didn't correlate well with adjacent FEC measurements, which they suggested may be linked to postweaning stress suppression of the acquired immune response. This pattern paralleled that reported by Bishop *et al.* (1996), but is probably not the reason for the low correlation between the two FEC reported here, as the faecal samples were collected immediately upon separation of the lamb from the ewe.

The heritability of FEC is reported to be low (0.2) at weaning in Merino sheep (Pollott *et al.* 2004), but increases to 0.7 at hogget age. However, the results from other studies summarised by Clarke (2002) show a more confusing pattern of heritability as lambs' age from weaning, and with no clear trend showing with age. Greeff *et al.* (1995) found that the heritability of FEC fell from 0.18 at weaning to 0.08 and then rose again to 0.25 at 12 mo of age. Greeff and Karlsson (1997) reported a similar trend with a value of 0.40 at weaning and 0.22 at hogget age. On the other hand, Greeff and Karlsson (1998) found similar FEC heritabilities at 3 and 15 mo of age. Recent industry data analyses of Merino sheep found the heritability of FEC to be 0.24 at yearling age and 0.38 at hogget age (M. Khurso, Armidale, Australia, unpublished data). Reports from other breeds suggest an increase in heritability of FEC with age (Morris *et al.*, 1997, 2000), and these reports from Romney sheep in New Zealand demonstrated a somewhat higher value for the heritability of FEC than reported by Pollott *et al.* (2004).

Pollott *et al.* (2004) conclude that the same genes largely control FEC at different ages. They argue this on the basis that as the repeatability of a trait gives an indication of the extent to which successive measurements of the trait on the same animals can be considered to be related to each other, then the difference between the repeatability and the heritability measures the permanent environmental effect of the animal on the trait plus any nonadditive effects.

In their work, the repeatability of the FEC data was 0.22, indicating they suggest that there is little relationship between successive FEC measurements other than those of genetic origin. The data to support this argument is however not shown.

However, Pollott *et al.* (2004) recognise that their analyses have not considered the effect that different species of parasitic worms may have had on the results. They report variation both between and within sites, at different times of the year, in the types of worm present, but there is no quantitative data on this aspect of FEC. If different species of worms produce different numbers of eggs per individual parasite, this will lead to variation in the results not considered by Pollott *et al.* (2004). There is evidence that there is a genetic correlation of 0.43 between strongyle (mainly *Trichostrongylus* and *Ostertagia*) and *Nematodirus* FEC (Morris *et al.* 2004), which they concluded indicates that the genetic mechanisms in sheep which are responsible for resistance to other strongyle nematodes probably also influence resistance to *Nematodirus* infection. Somewhat ironically Pollott *et al.* (2004) cite the work of Morris *et al.* (2004) but conclude that different genes and thus different defence mechanisms may operate against different parasite species. Pollott *et al.* (2004) conclude that more detailed recording of worm species needs to be undertaken in order to elaborate on this point.

This issue is of significance when considering the relationship between DQA2 and FEC for different parasites as detailed in this study. Specifically the results of both the within sire segregation analysis and the within farm general linear model suggest that weaning *Nematodirus spp.* faecal egg counts are linked to variation in the DQA2 gene. **These results would appear to be very reliable as the general linear model predicts an effect that is seen across three different farms, with three different breeds of sheep and with three different parasite profiles and levels of parasite species present.**

This finding is in accord with other findings linking the MHC with variation in resistance to parasites (Schwaiger *et al.* 1995, Stear *et al.* 1996, Buitkamp *et al.* 1996, Paterson *et al.* 1998) and supports the contention of Pollott *et al.* (2004) that more detailed recording of worm species needs to be undertaken in deciding the role of genes in resistance to parasites. Equally however, the DQA2 gene does not appear to be linked with autumn faecal egg counts for *Nematodirus spp.* While this result could support the argument that a different gene (or genes) is involved in according resistance to parasites in older lambs, which would contradict the conclusions of Pollott *et al.* (2004), it is interesting to note that the general linear models for describing the relationship between variation in faecal egg count for *Strongyle* parasites and DQA2 are also tending towards significance on two of the three properties and are significant on the Wilson property. It is therefore conceivable that the MHC is also involved in the response to *Strongyle* parasites, but at a later stage of lamb development.

This contention is consistent with the findings of Thomas (1959a,b) who showed that young grazing lambs appear to have the ability to limit *Nematodirus battus* egg production within a relatively short period of first being exposed to this parasite, although the findings of Israf *et al.* 1997 would suggest that after a prolonged period of exposure to *N. battus* (42 days), antibody titres, indicative of a specific immune response were low, although a relatively low dose (30 000 L<sub>3</sub> larvae) of *N. battus* was administered. This contrasts the findings of Sinclair *et al.* (1985) who showed a preponderance of IgA-containing cells when *N. battus* is present in abundance. Regardless, the role of Class II MHC in initiating antibody responses cannot be overlooked and hence our finding of a link between DQA2 and *Nematodirus spp.* faecal egg counts at weaning suggests that the acquisition of specific immunity may be a key regulator of *Nematodirus spp.* faecal egg counts and that specific alleles of DQA2 may “mark” lambs with reduced egg output. Confirming this finding will require further research with greater numbers of lambs of any given DQA2 genotype.

## Conclusions from this Project

- Drench resistance was emerging on all properties to all drenches with the exception of clear drenches.
- The only conclusion in regard to species mix of parasites in any given parasite challenge on a property, is that there is no precise way of knowing the species present other than to measure.
- There are significant sire-line differences in the mean FEC of progeny which could be of use to farmers in breeding.
- There is an association between the DQA2 gene and FEC for *Nematodirus spp.* at weaning both within and across sire-lines on a given property, but the precise nature of this association on an allele by allele basis will require the analysis of many more sheep.

## Future Work

Further work is also being undertaken by Lincoln University to look at a new potential gene marker for parasite resistance using this data set as a basis for that research. Early results are very promising

We have also started to put this data through both Lambplan and SIL so that sires can be ranked and EBV'S generated. This meant that the project was not completed until late 2004.

John Bates  
Jon Hickford  
February 2005

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