Major Histocompatibility Complex DRB1 gene: its role in nematode resistance in Suffolk and Texel sheep breeds

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SUMMARY

A potential control strategy for nematode infection in sheep is the implementation of a breeding programme to select for genes associated with resistance. The Texel breed is more resistant to gastrointestinal nematode infection than the Suffolk breed, based on faecal egg count, and this difference should enable the identification of some of the genes responsible for resistance. The objective of this study was to determine if variation at the ovine MHC-DRB1 locus was associated with variation in faecal egg count in Suffolk and Texel sheep. Ovar-DRB1 alleles and faecal egg count were determined for Texel ($n=105$) and Suffolk ($n=71$) lambs. Eight Ovar-DRB1 alleles, including 1 previously unknown allele, were identified in the Texel breed by sequence-base-typing. Seven Ovar-DRB1 alleles were identified in the Suffolk breed. Two Ovar-DRB1 alleles were common to both breeds, but were among the least frequent in the Suffolk population. In the Suffolk breed 1 Ovar-DRB1 allele was associated with a decrease in faecal egg count and 2 alleles with an increase in faecal egg count. This locus accounted for 14% of the natural variation in faecal egg count in Suffolks. There was no evidence for an association between Ovar-DRB1 alleles and faecal egg count in the Texel breed and the Ovar-DRB1 locus accounted for only 3% of the phenotypic variation in faecal egg count. These results suggest that the Ovar-DRB1 gene plays an important role in resistance to nematode infection in the Suffolk breed. The difference in faecal egg counts between these breeds may be attributable in part to the different allele profile at the Ovar-DRB1 locus.

Key words: Ovar-DRB1, faecal egg count, disease resistance, sheep, parasites.

INTRODUCTION

Nematode species of the genus *Teladorsagia* (previously *Ostertagia*), *Trichostrongylus* and *Nematodirus* are largely responsible for gastrointestinal parasitic infections in sheep in Ireland (Good *et al*. 2004). The search for control measures to supplement anthelmintics in controlling these endoparasites in sheep has involved a number of different approaches. These include the use of tanniferous forages (Niezen *et al*. 1998), protein supplementation (Datta *et al*. 1999; Coop and Kyriazakis, 2001), the development of nematode vaccines (Andrews *et al*. 1995; Andrews, Rolph and Munn, 1997; Knox *et al*. 2003), the use of nematophagus fungi to reduce larval numbers on pasture (Githigia *et al*. 1997; Larsen *et al*. 1998; Pena *et al*. 2002) and the replacement of anthelmintic- resistant nematodes with those of a susceptible genotype (Bird *et al*. 2001). However, the proven success of genetic selection for nematode resistance based on faecal egg count (FEC) has highlighted the potential of this method of nematode control (Eady, Woolaston and Barger, 2003; Kahn *et al*. 2003). Selective breeding for resistance diminishes the impact of infection on production, lowers the requirement for anthelmintic control and reduces larval pasture contamination (Bisset *et al*. 1997). Identifying loci associated with natural resistance would facilitate selection of resistant animals and aid the understanding of host-parasite interactions. Genes located within the ovine major histocompatibility complex (MHC) class I and II regions are strong candidates for resistance because of their central role in antigen presentation to T cells and their associations with nematode resistance (Hulme *et al*. 1991; Buitkamp *et al*. 1996; Outteridge *et al*. 1996; Stear *et al*. 1996). The MHC class I and II molecules are membrane-bound glycoproteins that bind foreign antigen in an antigenic cleft for display to T cells. Exon 2 of the Ovar-DRB1 gene codes for part of the MHC class II antigen binding cleft and in sheep over 80 alleles have been identified at this locus. In the Scottish Blackface breed one exon 2 allele has been associated with a significant reduction in FEC (Schwaiger *et al*. 1995). A number of different methods have been published for Ovar-DRB1 genotyping including direct cloning followed by sequencing (Konnai *et al*. 2003*a*), immunoprinting (Schwaiger *et al*. 1993), PCR-Restriction Fragment Length Polymorphism (Blattman *et al*. 1993; Grain *et al*. 1993; Konnai *et al*.

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2003*b*) and Single Strand Conformational Polymorphism (Outteridge *et al*. 1996; Kostia *et al*. 1998).

Studies in Ireland have shown that the Texel breed is more resistant to nematode infection than co-grazed Suffolks, based on FEC measurements and worm burdens (Hanrahan and Crowley, 1999; Good *et al*. 2004). As part of a project to explain the basis for this genetic difference in nematode resistance in sheep, the allelic variation at exon 2 of the Ovar-DRB1 locus was assessed in order to determine the role of this gene in genetic resistance to nematode infection.

Table 1. Mean (± S.E.) faecal egg counts for Texel and Suffolk lambs at 17 weeks of age over 4 years, 1999 to 2002, on the ln (x + FEC) and observed scales

	FEC	
Breed	Mean ± S.E. on ln (x + FEC) scale	95% confidence interval of mean on the observed scale
Texel	5·7 ± 0·10	188 to 304
Suffolk	6·6 ± 0·11	565 to 891
F-test	$P < 0·001$	—

MATERIALS AND METHODS

Animals

A total number of 155 Suffolk and 179 Texel pure-bred lambs were available for the study. These lambs represented 4 annual lamb crops (1999–2002). Texel lambs were derived from 11 sires and 50 different dams, Suffolk lambs were derived from 11 sires and 43 different dams. Sires were not used to produce the dams. All lambs were born in early March each year and co-grazed on pasture from birth to weaning. A single anthelmintic treatment (levamisole or benzimadazole, depending on the year) was administered at 5 weeks of age for the control of *Nematodirus*. Lambs were weaned at 17 weeks of age.

Faecal egg count

Faecal samples were obtained from the rectum of Suffolk (n = 120) and Texel (n = 148) lambs at 17 weeks of age and a 3 g aliquot of each faecal sample was processed to determine the number of worm eggs per gram of faeces (FEC) using the modified McMaster method (MAFF, 1986). Eggs of *Nematodirus* spp. were enumerated separately from those of other trichostrongyle species. As numbers of *Nematodirus* were extremely low they were not included in the analysis.

Ovar-DRB1 genotyping

Blood samples were collected in EDTA vacutainers from lambs born from 2000 to 2002. DNA was extracted from blood using a lysis buffer and proteinase-K digestion technique. For the 1999-born lambs, a ground sample of meat was the only source of DNA but the poor quality of this DNA meant that genotype analysis was unreliable for this gene. Consequently Ovar-DRB1 genotype could only be established for 87 Suffolks and 111 Texels.

Sequence-base-typing, validated by cloning, was the method of genotyping employed in this study. Sequence-base-typing is a technique whereby each Ovar-DRB1 gene was amplified by PCR, sequenced, and alleles identified from the chromatogram. This was the technique of choice because it provided a sequence of the exon for each animal, thereby highlighting any potential single-base mutations which may be missed by other methods of analysis.

Approximately 100 ng of DNA was amplified in a 50 μl polymerase chain reaction (PCR) with 2·5 U *Taq* polymerase (Promega Southampton, UK). Amplification was carried out for 30 cycles in a Peltier Thermal Cycler (MJ Research, Massachusetts, USA). A cycle consisted of a 30 s denaturation at 94 °C, a 30 s annealing at 57 °C and a 1 min extension at 72 °C. A forward primer (5′-TCTCTGCAGCACATTTCCTGG-3′) (adopted from Schwaiger *et al*. 1995) and reverse primer (5′-CACACACACACTGCTCCACA-3′) (in-house designed primer) pair were employed to amplify the second exon of the Ovar-DRB1 gene, yielding a 308 base-pair product. These primers were located in intron 1 and 3 respectively thereby flanking the exon 2 region. PCR products were cleaned using Microcon® YM-100 centrifugal filter devices (Millipore, Massachusetts, USA) and DNA concentration determined by comparison with a low mass DNA ladder (Invitrogen, Life Technologies, California, USA). Approximately 8 ng PCR product was sequenced using the reverse primer for the Ovar-DRB1 gene with the ABI Prism® BigDye™ Terminator reaction kit version 2. Sequencing reactions were run on an ABI Prism® 377 DNA sequencer.

Sequences were viewed using Chromas software version 1.45 (Technelysium Ltd, Queensland, Australia). The identity of each Ovar-DRB1 allele was determined using custom written software (SMS Software, T. Bellenger). This software compared the query sequence to an in-house generated library of ovine DRB1 alleles and identified the alleles for which it is an exact match. This library contained 88 different allele sequences available from Genbank. The operating principle is similar to that of 'BLAST', except the library is self-generated, it can accommodate heterozygous sequences and does not offer a 'best fit' scenario but an exact match only. Allele identification from

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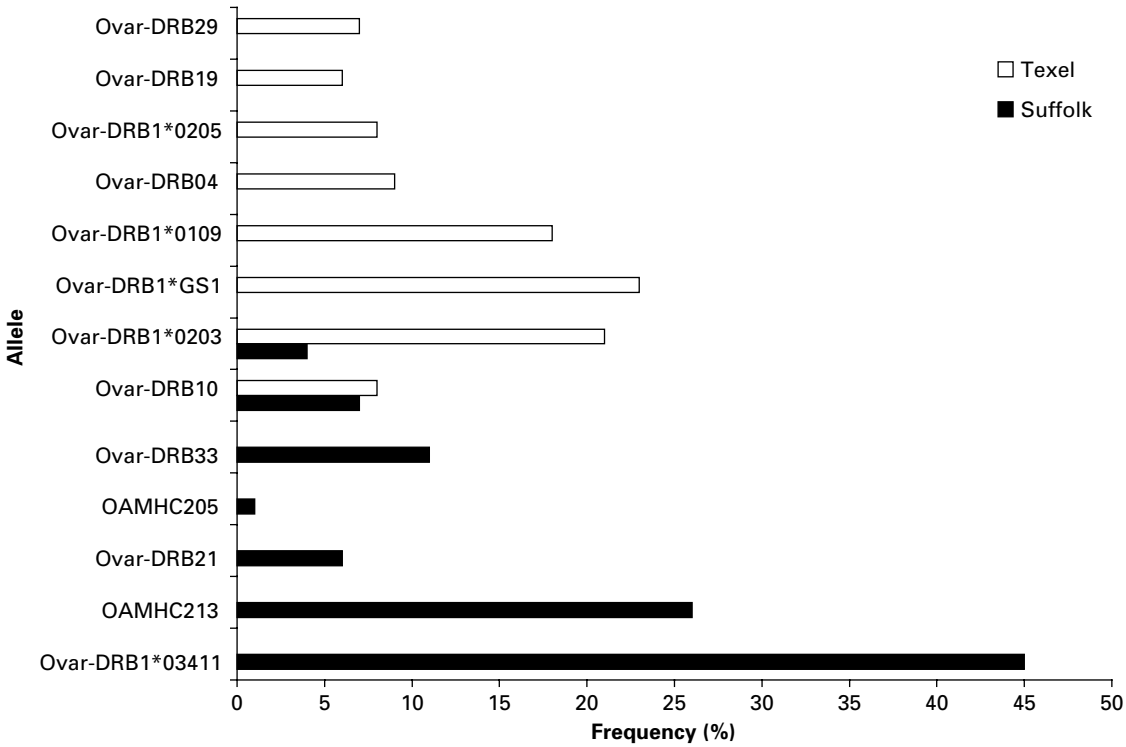

Fig. 1. Ovar-DRB1 allelic frequency in Texels ($n=111$) and Suffolks ($n=87$). Alleles were identified by computer comparison to a library of Ovar-DRB1 alleles.

sequences resulting from cloning or from homozygous animals was clear-cut. Where the sequence obtained represented 2 different alleles it was first edited and heterozygous positions coded using the International Union of Biochemistry nucleotide translation conventions (http://www.chem.qmul.ac.uk/iupac/): M = A + C, S = C + G, R = A + G, Y = C + T, W = A + T, K = G + T. This convention is recognized by the software, which then generates all possible alleles from the heterozygous query sequence. The 2 alleles that are an exact match for the query sequence are identified. With approximately 20 potential polymorphic positions within a heterozygous sequence, the chance of 2 sets of alleles matching the query sequence is highly unlikely and did not occur. If it did, the programme would offer both sets.

In order to confirm the reliability of this procedure 3 Suffolk and 3 Texel sires, those with the largest progeny numbers, had their Ovar-DRB1 gene amplified by PCR and cloned using the TOPO TA Cloning® kit (Invitrogen Life Technologies, California, USA). To amplify the insert, PCR was carried out on DNA extracted from growing cultures using the M13 primer set. Sequencing of the insert was completed using the M13 forward and reverse primers.

Statistical analysis

The FEC values were transformed to ln (FEC + 25) prior to analysis and only the FEC values based on eggs from Trichostrongyles (excluding *Nematodirus* species) were considered. As numbers of *Nematodirus* were low in both breeds, this species were not considered further. Preliminary analyses were done using Proc GLM of SAS (SAS, 1989) to fit a linear model with effects for breed, year of birth (1999–2002), sample date within year, rearing type (single or twin) and sex and breed by year interaction. A full animal model, with a pedigree file going back to the foundation of the flocks in the mid-1970s, was used to estimate the effects of the Ovar-DRB1 alleles on FEC. The allele effects were evaluated by adding regression terms to the model for each allele except the most common allele in each breed. This analysis was carried on a within breed basis, because of the difference in allele profile between the breeds, and was done using the PEST software (Groenveld, 1990). All available FEC data were included and a heritability of 0·25 was used in both breeds. In total, 71 Suffolks and 105 Texels were used in the analysis as these animals had both genotype information and FEC measurements available. The difference among the regression coefficients obtained for the alleles were evaluated using Duncan's multiple range test (Steele and Torrie, 1960).

RESULTS

Faecal egg count analysis

Mean FEC for all Suffolk and Texel lambs are presented in Table 1, which show that Suffolk

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                9         19         29         39         49         59         69         79         89
Ovar-DRB01      YTKKECRFS NGTERVRFLD RYFHNGEETL RFDSDWGEYR AVAELGRPDA KYWNSQKDFL ERARAAVDTY CRHNYGVIES FTVQRR
Ovar-DRB1*GS1   ------H-F -------L-E --------YV --------F- -------QS- E------EI- -SR-T----- ---------- -S----
Ovar-DRB04      --------- ---------- ---------- ---------- ---------- ---------- ---------- -------G-- -S----
Ovar-DRB10      -STS----F ---------- ---Y----YV ---------- ---------- -------EI- --R-TE---- ---------- -S----
Ovar-DRB19      -A-S----F ---------E ---Y----YV ---------- ---------- ---------- --K--N---- -------G-- ------
Ovar-DRB29      -H-S--H-F ---------- ---Y----YV --------F- --------E- -------EI- --R-TE---- ---------- -S----
Ovar-DRB1*0109  --------F -------Y-- ---Y----YV ---N------ ---------- -------EL- --K--N---- -------G-- ------
Ovar-DRB1*0203  -STS--H-F ---------- ---Y------ ---------- ---------- -------EI- --K------- -------G-- ------
Ovar-DRB1*0205  -STS--H-F ---------- ---Y----YV ---------- ---------- -------EI- --R-TE---- -------F-- ------
Ovar-DRB1*03411 -H-S----F ---------- ---Y----YV --------F- ---------- -------EI- --K------- ---------- -S----
OAMHC213        -S-S--H-F ---------- --YT----NV --------F- --T------- EQ-------- -SR-T----- -------F-- -S----
Ovar-DRB21      -H-S--H-F ---------- --------FV --------F- -------RS- E------EL- --R-TE---- -------F-- -S----
Ovar-DRB33      -YRS--H-F -------L-E --------FA --------F- --T-----A- EQ-----NI- -QK--E-N-V -------F-- -A----
OAMHC205        -R-S----- ---------- ---Y----NV ---------- ---------- ---------- --K--N---- -------G-- ------
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Fig. 2. Protein translation of the second exon of Ovar-DRB1 alleles investigated for associations with resistance to nematode infection in this study. Each protein translation is displayed relative to Ovar-DRB01 (Accession no. U00204).

lambs had significantly ($P<0{\cdot}001$) higher egg output than Texel lambs. The statistical analysis also showed that year and sampling date had highly significant effects ($P<0{\cdot}001$ in both cases) and that the rearing type effect approached significance ($P=0{\cdot}07$). There was no evidence of a significant year by breed interaction. The effect for sex did not approach significance and was not included in the mixed models used to estimate the allele effects.

Ovar-DRB1 alleles

Sequence-based-typing of lambs yielded a total of 8 alleles in Texels and 7 in Suffolks. The frequency and protein translation of each allele is shown in Figs 1 and 2 respectively. Only 2 alleles were common to both breeds and these were among the least frequent alleles in Suffolks. One previously unidentified allele was found in the Texel breed (Ovar-DRB1*GS1, Genbank Accession no. AY227049).

The sequencing was initially performed in 2 directions in all the sires in which the Ovar-DRB1 gene was cloned. In the 3 Texel sires investigated 5 alleles were identified (Ovar-DRB1*0109, Ovar-DRB1*0203, Ovar-DRB29, Ovar-*DRB*10, Ovar-DRB1*GS1). In the 3 Suffolk sires investigated 3 alleles were identified (Ovar-DRB33, OAMHC213, Ovar-DRB1*03411). The results from cloning confirmed the validity of the Ovar-DRB1 sequence-base-typing analysis method. Thus, sequencing was only carried out in 1 direction for all of the offspring. Without exception, 1 of the alleles carried by a sire was identified in each of its offspring. To re-confirm that the correct allele was being identified, linkage to an adjacent intronic microsatellite was also investigated, a method previously described by Schwaiger *et al*. (1995). Again, allele designation was consistent in all cases (results not shown).

Effect of Ovar-DRB1 variation on FEC

The estimated effects of allele substitution are given in Tables 2 and 3 for Suffolks and Texels, respectively. The effect for Ovar-*DRB*1*0203 just failed to reach formal statistical significance ($P=0{\cdot}052$) while those for Ovar-DRB33 and OAMHC213 were associated with *P*-values of about 0·12. The substitution of the most common allele in the Suffolk breed, Ovar-DRB1*03411, with Ovar-*DRB*1*0203 led to a large decrease (−0·81) in log-transformed FEC, relative to the most common allele. On the untransformed scale this represents a 45% reduction while the substitution of Ovar-DRB1*03411 with Ovar-DRB33 reduced FEC by approximately 30% on the untransformed scale. The substitution of Ovar-DRB1*03411 by either OAMHC213 or Ovar-DRB10 increased FEC by about 25%. The substitution effects for OAMHC205 was not analysed because only 1 copy of this allele was present in the sample. Evaluation of the differences among the allele effects showed that the effects of Ovar-*DRB*1*0203 and Ovar-DRB33 were both significantly different from the effect of OAMHC213 ($P<0{\cdot}05$).

In Texels none of the allele substitution effects, relative to the most common allele Ovar-DRB1*GS1, were statistically significant.

There was no significant effect for heterozygosity in either breed. In Texels 14% of animals were homozygous and had a transformed FEC of $6{\cdot}20\pm0{\cdot}325$, the corresponding figure for heterozygous Texels was $5{\cdot}57\pm0{\cdot}163$. In Suffolks, 35% of animals were homozygous and had transformed FEC of $6{\cdot}71\pm0{\cdot}271$, heterozygous Suffolks had a mean transformed FEC of $6{\cdot}72\pm0{\cdot}187$.

Variation at the Ovar-DRB1 locus accounted for 14% of the phenotypic variation in Suffolks (after adjusting for the fixed effects of year, sample date and rearing type). The corresponding figure in Texels was 3%.

DISCUSSION

The populations of Suffolk and Texel sheep examined in this study exhibited low variation at the Ovar-DRB1 locus compared with results from other studies. Schwaiger *et al*. (1995) identified 19 alleles in a sample of 299 Scottish Blackface animals while Konnai *et al*. (2003*a*) found 28 alleles among

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Table 2. Substitution effects (relative to the most common allele – DRB1*03411) for alleles at the Ovar-DRB1 locus on faecal egg count (ln (FEC + 25) scale) in Suffolks

Allele	Genbank Accession number	Allele substitution effect	S.E.	Probability
Ovar-DRB1*03411	AB017218	—	—	—
OAMHC213	Y10246	0·26[b]	0·17	<0·12
Ovar-DRB10	U00212	0·21[ab]	0·33	>0·5
Ovar-DRB21	U00224	0·08[ab]	0·27	>0·7
Ovar-DRB33	U00236	−0·36[a]	0·23	<0·12
Ovar-DRB1*0203	AB017206	−0·81[a]	0·41	=0·05

[ab] Estimates without a superscript letter in common are significantly different ($P<0·05$).

Table 3. Substitution effects (relative to the most common allele – Ovar-DRB1*GS1) for alleles at the Ovar-DRB1 locus on faecal egg count (ln (FEC + 25) scale) in Texels

Allele	Genbank Accession number	Allele substitution effect	S.E.	Probability
Ovar-DRB1*GS1	AY227049	—	—	—
Ovar-DRB04	U00206	−0·14	0·31	>0·6
Ovar-DRB10	U00212	−0·15	0·32	>0·6
Ovar-DRB19	U00222	−0·08	0·35	>0·8
Ovar-DRB29	U00232	−0·08	0·34	>0·8
Ovar-DRB1*0109	AB017209	−0·12	0·25	>0·6
Ovar-DRB1*0203	AB017206	−0·28	0·22	=0·20
Ovar-DRB1*0205	AF036560	−0·06	0·28	>0·8

71 Suffolks, 14 alleles from 20 Cheviots and 9 alleles from 6 Corriedale sheep. While the Suffolk and Texel flocks used in the present study represent small self-contained lines the average inbreeding coefficients for the cohorts used were only 7·7% and 7·1%, respectively. Thus a small effective population size is unlikely to account for the relatively low number of alleles present. The slightly higher allelic variation in the Texel compared with the Suffolk is expected and is consistent with other findings (Walling *et al*. 2004).

It has been suggested that homozygosity at the Ovar-DRB1 locus may impair ability to respond effectively to diseases (Thursz *et al*. 1997; Sauermann *et al*. 2000; M. Stear, personal communication). However, in this study there was no evidence for any significant effect of heterozygosity at the Ovar-DRB1 locus on FEC (homozygotes: $6·46 \pm 0·227$; heterozygotes $6·14 \pm 0·136$, $P=0·26$).

In the Suffolk population examined here the Ovar-DRB1 gene accounted for 14% of the phenotypic variation in FEC and the evidence indicated that 3 alleles were largely responsible for this variation. This finding is consistent with disease association studies in the Scottish Blackface breed, where the Ovar-DRB1 gene accounted for 11% of the phenotypic variation in FEC and 1 Ovar-DRB1 allele was associated with a reduced faecal egg count (Schwaiger *et al*. 1995). It is interesting to note that the allele associated with reduced FEC in the present Suffolk population is the same allele as that associated with reduced FEC in the Scottish Blackface breed (M. Stear, personal communication).

The absence of an association between Ovar-DRB1 alleles and FEC in Texels is consistent with that of Blattman *et al*. (1993) who were unable to detect any evidence of an effect of the DQB or DRB regions of the MHC complex on resistance in the Merino breed. A number of explanations may be offered for the absence of evidence for an effect of the Ovar-DRB1 gene on FEC in Texels. These include linkage disequilibrium to a resistant locus that is established in Suffolks but not in Texels (linkage disequilibrium has been reported between MHC class I and II loci (Stear *et al*. 1996)) or favourable haplotypic combinations to combat infection which are present in Suffolks but not in Texels or age-related effects whereby the role of the Ovar-DRB1 allele is evident in Texels at ages other than 17 weeks (age-related effects previously reported by Schwaiger *et al*. 1995). However, it is most likely that since the Texel breed has a very different profile of alleles at the Ovar-DRB1 locus and the fact that the resistant allele in Suffolks is the second-most frequent in Texels, the majority of Ovar-DRB1 alleles in Texels are associated with resistance to nematodes. This would account for the observed

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breed difference in FEC and also explain why no one allele was associated with resistance in this breed.

The DRB1 exon 2 product forms part of the MHC class II antigen-binding cleft and amino acids in this region determine the size, form and charge of the cleft and, more importantly, the side-chains of foreign antigen that may be accommodated in the cleft (Stern *et al*. 1994; Thorsby, 1999). Thus, some Ovar-DRB1 alleles may be better suited to display antigens common to certain diseases and so generate a better immune response through a better T-cell response repertoire (Krieger *et al*. 1991; Coppin *et al*. 1993). It is argued that alleles associated with resistance, as shown in this study and by Schwaiger *et al*. (1995) possess the necessary amino acid combination to facilitate an effective immune challenge to combat nematode infection.

The evidence from this study together with the allele effects previously found in the Scottish Blackface breed support the hypothesis that Ovar-DRB1 gene plays an important role in the reduced resistance of Suffolk sheep to parasite infection. Further studies with larger animal numbers are desirable to confirm the present results in this breed and the utility of selection based on the genetic variation described. Specific mating plans will have to be implemented to directly establish the role of the Ovar-DRB1 gene in the large difference in FEC between Texel and Suffolk animals and, by implication, in resistance. The amino acid changes associated with the alleles shown to affect resistance support the view that these changes affect functionality of the MHC class II protein product.

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REFERENCES

Andrews, S. J., Hole, N. J. K., Munn, E. A. and Rolph, T. P. (1995). Vaccination of sheep against haemonchosis with H11, a gut membrane-derived protective antigen from the adult parasite: prevention of the periparturient rise and colostral transfer of protective immunity. *International Journal for Parasitology* **25**, 839–846.

Andrews, S. J., Rolph, T. P. and Munn, E. A. (1997). Duration of protective immunity against ovine haemonchosis following vaccination with the nematode gut membrane antigen H11. *Research in Veterinary Science* **62**, 223–227.

Bird, J., Shulaw, W. P., Pope, W. F. and Bremer, C. A. (2001). Control of anthelmintic resistant endoparasites in a commercial sheep flock through parasite community replacement. *Veterinary Parasitology* **97**, 219–225.

Bisset, S. A., Vlassoff, A., West, C. J. and Morrison, L. (1997). Epidemiology of nematodosis in Romney lambs selectively bred for resistance or susceptibility to nematode infection. *Veterinary Parasitology* **70**, 255–269.

Blattman, A. N., Hulme, D. J., Kinghorn, B. P., Woolaston, R. R., Gray, G. D. and Beh, K. J. (1993). A search for associations between major histocompatibility complex restriction fragment length polymorphism bands and resistance to *Haemonchus contortus* infection in sheep. *Animal Genetics* **24**, 277–282.

Buitkamp, J., Filmether, P., Stear, M. J. and Epplen, J. T. (1996). Class I and class II major histocompatibility complex alleles are associated with faecal egg counts following natural, predominantly *Ostertagia circumcincta* infection. *Parasitology Research* **82**, 693–696.

Coop, R. L. and Kyriazakis, I. (2001). Influence of host nutrition on the development and consequences of nematode parasitism in ruminants. *Trends in Parasitology* **17**, 325–330.

Coppin, H. L., Carmichael, P., Lombardi, G., L'faqihi, F. E., Salter, R., Parham, P., Lechler, R. I. and de Preval, C. (1993). Position 71 in the alpha helix of the DR beta domain is predicted to influence peptide binding and plays a central role in allorecognition. *European Journal for Immunology* **23**, 343–349.

Datta, F. U., Nolan, J. V., Rowe, J. B., Gray, G. D. and Crook, B. J. (1999). Long-term effects of short-term provision of protein-enriched diets on resistance to nematode infection and live-weight gain and wool growth in sheep. *International. Journal for Parasitology* **29**, 479–488.

Eady, S. J., Woolaston, R. R. and Barger, I. A. (2003). Comparison of genetic and nongenetic strategies for control of gastrointestinal nematodes of sheep. *Livestock Production Science* **81**, 11–23.

Githigia, S. M., Thamsborg, S. M., Larsen, M., Kyvsgaard, N. C. and Nansen, P. (1997). The preventative effect of the fungus *Duddingtonia flagrans* on trichostrongyle infections of lambs on pasture. *International Journal for Parasitology* **27**, 931–939.

Good, B., Hanrahan, J. P., Crowley, B. A. and Mulcahy, G. (2004). Texel sheep are more resistant than Suffolks to natural helminth challenge based on faecal egg count and nematode burden. Manuscript submitted to *Parasitology*.

Grain, F., Nain, M.-C., Labonne, M.-P., Lantier, F., Lechopier, P., Gebuhrer, L., Asso, J., Maddox, J. and Betuel, H. (1993). Restriction fragment length polymorphism of DQB and DRB class II genes of the ovine major histocompatibility complex. *Animal Genetics* **24**, 377–384.

Groeneveld, E. (1990). *PEST User's Manual, Version 3.1*.

Hanrahan, J. P. and Crowley, B. A. (1999). Evidence for breed differences in resistance to nematode parasitism. *Sheep and Goat Commission of European Association for Animal Production* (*50th Annual Meeting*) Session IV, 4 pp.

Hulme, D. J., Windon, R. G., Nicholas, F. W. and Beh, K. J. (1991). Association between MHC Class II RFLP and *Trichostrongylus* resistance. In *Breeding for Disease Resistance in Sheep* (ed. Gray, G. D. &

Woolaston, R. R.), pp. 115–120. Australian Wool Corporation, Melbourne.

Kahn, L. P., Knox, M. R., Gray, G. D., Lea, J. M. and Walkden-Brown, S. W. (2003). Enhancing immunity to parasites in single-bearing Merino ewes through nutrition and genetic selection. *Veterinary Parasitology* **112**, 211–225.

Knox, D. P., Redmond, D. L., Newlands, G. F., Skuce, P. J., Pettit, D. and Smith, W. D. (2003). The nature and prospects for gut membrane proteins as vaccine candidates for *Haemonchus contortus* and other ruminant trichotrongyloids. *International Journal for Parasitology* **33**, 1129–1137.

Konnai, S., Nagaoka, Y., Takesima, S., Onuma, M. and Aida, Y. (2003*a*). Sequences and diversity of 17 new Ovar-DRB1 alleles from three breeds of sheep. *European Journal for Immunogenetics* **30**, 275–282.

Konnai, S., Nagaoka, Y., Takesima, S., Onuma, M. and Aida, Y. (2003*b*). Technical note: DNA typing for ovine MHC DRB1 using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). *Journal of Dairy Science* **86**, 3362–3365.

Kostia, S., Kantanen, J., Kolkkala, M. and Varvio, S. L. (1998). Applicability of SSCP analysis for MHC genotyping: fingerprinting of Ovar-DRB1 exon 2 alleles from Finnish and Russian breeds. *Animal Genetics* **29**, 453–455.

Krieger, J. I., Karr, R. W., Grey, M., Yu, W.-Y., O'Sullivan, D., Batovsky, L., Zheng, Z.-L., Colon, S. M., Gaeta, F. C. A., Sidney, J., Albertson, M., Del Guercio, M.-F., Chesnut, R. W. and Sette, A. (1991). Single amino acid changes in DR and antigen define residues critical for peptide-MHC binding and T cell recognition. *Journal of Immunology* **146**, 2331–2340.

Larsen, M., Faedo, M., Waller, P. J. and Hennessy, D. R. (1998). The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep: Studies with *Duddingtonia flagrans*. *Veterinary Parasitology* **76**, 121–128.

Ministry of Agriculture, Fisheries and Food (1986) *Manual of Veterinary Parasitological Laboratory Techniques, 3rd Edn*.

Niezen, J. H., Robertson, H. A., Waghorn, G. C. and Charleston, W. A. (1998). Production, faecal egg counts and worm burdens of ewe lambs which grazed six contrasting forages. *Veterinary Parasitology* **80**, 15–27.

Outteridge, P. M., Andersson, L., Douch, P. G., Green, R. S., Gwakisa, P. S., Hohenhaus, M. A. and Mikko, S. (1996). The PCR typing of MHC-DRB genes in the sheep using primers for an intronic microsatellite: application to nematode parasite resistance. *Immunology and Cell Biology* **74**, 330–336.

Pena, M. T., Miller, J. E., Fontenot, M. E., Gillespie, A. and Larsen, M. (2002). Evaluation of *Duddingtonia flagrans* in reducing infective larvae of *Haemonchus contortus* in faeces of sheep. *Veterinary Parasitology* **103**, 259–265.

SAS (1989). *SAS/STAT User's Guide, Version 6, Fourth Edition, Vol. 2*. SAS Institute Inc., Cary, NC, USA.

Sauermann, U., Stahl-Hennig, C., Stolte, N., Muhl, T., Krawczak, M., Spring, M., Fuchs, D., Kaup, F. J., Hunsmann, G. and Sopper, S. (2000). Homozygosity for a conserved MHC class II *DQ-DRB* haplotype is associated with rapid disease progression in simian immunodeficiency virus-infected macaques: results from a prospective study. *Journal of Infectious Diseases* **182**, 716–724.

Schwaiger, F.-W., Buitkamp, J., Weyers, E. and Epplen, J. T. (1993). Typing of the artiodactyl MHC-*DRB* genes with the help of intronic simple repeated DNA sequences. *Molecular Ecology* **2**, 55–59.

Schwaiger, F.-W., Gostomski, D., Stear, M. J., Duncan, J. L., McKellar, Q. A., Epplen, J. T. and Buitkamp, J. (1995). An ovine major histocompatibility complex DRB1 allele is associated with low faecal egg counts following natural, predominantly *Ostertagia circumcincta* infection. *International Journal for Parasitology* **25**, 815–822.

Stear, M. J., Bairden, K., Bishop, S. C., Buitkamp, J., Epplen, J. T., Gostomski, D., McKellar, A. Q., Schwaiger, F.-W. and Wallace, D. S. (1996). An ovine lymphocyte antigen is associated with reduced faecal egg counts in four-month-old lambs following natural, predominantly *Ostertagia circumcincta* infection. *International Journal for Parasitology* **26**, 423–428.

Steel, R. G. D. and Torrie, J. H. (1960). *Principles and Procedures of Statistics*. McGraw-Hill Book Company Inc., New York.

Stern, L. J., Brown, J. H., Jardetzky, T. S., Gorga, J. C., Urban, R. G., Strominger, J. L. and Wiley, D. C. (1994). Crystal structure of the human class II MHC protein HLA-*DR1* complexed with an influenza virus peptide. *Nature, London* **368**, 215–221.

Thorsby, E. (1999). MHC structure and function. *Transplantation Proceedings* **31**, 713–716.

Thursz, M. R., Thomas, H. C., Greenwood, B. M. and Hill, A. V. (1997). Heterozygote advantage for HLA class-II type in hepatitis B virus infection. *Nature Genetics* **17**, 11–12.

Walling, G. A., Wilson, A. D., McTeir, T. L. and Bishop, S. C. (2004). Increased heterozygosity and allelic variants are seen in Texels compared to Suffolk sheep. *Heredity* **92**, 102–109.