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Intron 1 of the interferon γ gene: Its role in nematode resistance in Suffolk and Texel sheep breeds

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Abstract

Genetic variation at intron 1 of the interferon γ gene has recently been associated with variation in faecal egg count in Romney, Merino and Soay breeds of sheep. The Texel breed is more resistant to gastrointestinal nematode infection than the Suffolk breed, based on faecal egg count. Hence, the objective of this experiment was to characterise the polymorphisms of intron 1 of the interferon γgene in Suffolk and Texel sheep, and to determine if the characterised haplotype variants were associated with faecal egg count variation in these breeds. Intron 1 haplotypes and faecal egg measurements were determined for Suffolks ($n = 113$) and Texels ($n = 135$). Four haplotypes were identified in Suffolks (A, B, C and D), two haplotypes as previously described, and two previously unknown haplotypes. However, there was no association between interferon γ intron 1 haplotype and faecal egg count in Suffolks. In contrast, two previously identified haplotypes (A and B) were present in the Texel breed, and the B haplotype was associated with resistance to nematode infection ($P = 0.02$). These results suggest that intron 1 of the interferon γ gene has an important role in resistance to nematode infection in the Texel breed.
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1. Introduction

Breeding for resistance to nematode infection provides an additional strategy to complement the use of anthelmintics in sheep husbandry practices. Resistant animals can be selected on the basis of low faecal egg count (FEC) (Eady et al., 2003; Kahn et al., 2003). A disadvantage of FEC as a marker of resistance is the requirement for animals to be infected, either naturally or artificially, to determine the FEC value and the effort and cost in obtaining FEC measurements. An alternative to FEC analysis is identifying and selecting for genes that are responsible for resistance to nematode infection. A number of genes have been associated with nematode resistance in sheep: the interferon γ (IFN-γ) gene (Coltman et al., 2001; Crawford and McEwan, 1999), the Major Histocompatibility Complex genes (Schwaiger et al., 1995; Buitkamp et al., 1996; Outteridge et al., 1996; Stear et al., 1996; Sayers et al., 2005), the 5′ end of the IgE gene (Clarke et al., 2001) and microsatellites located near the interleukin (IL) -3, -4 and -5 genes (Benavides et al., 2002).

The IFN-γ gene has received increased attention because of its association with nematode resistance in both domestic and free-living sheep breeds. Crawford and McEwan (1999) (as cited by Coltman et al., 2001) examined four markers on ovine chromosome 3, which included one microsatellite marker located in intron 1 of the IFN-γ gene. Association studies highlighted an important role for this microsatellite marker in nematode resistance in the Romney and Merino breeds (Crawford

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and McEwan, 1999). The importance of this microsatellite was again demonstrated by its association with nematode resistance in the feral Soay breed (Coltman et al., 2001). This microsatellite has been characterised as a bi-allelic (GTTT) repeat, allele A has six repeats and allele B, five repeats (Schmidt et al., 1996). In addition, allele A has a 'G' 49 bp downstream of the microsatellite (refered to as haplotype A) while allele B has an 'A' at the corresponding position (refered to as haplotype *B*) (GenBank Accession No. Z73273). In both studies, the B haplotype was associated with resistance to nematode infection as outlined by Coltman et al. (2001).

In Ireland, the Texel breed has been shown to be more resistant to nematode infection than co-grazed Suffolks, based on FEC measurements (Hanrahan and Crowley, 1999). As part of a project to explain this difference in resistance between purebred Suffolks and Texels, the haplotypic variation at intron one of the IFN-γ gene was determined and its association with nematode resistance in these breeds was assessed.

2. Materials and methods

2.1. Animals

A total of 155 Suffolk and 179 Texel purebred lambs were available for the study. These lambs represented four annual lamb crops (1999–2002) and were derived from 13 Texel and 17 Suffolk sires. 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 about 5 weeks of age for the control of *Nematodirus battus*. Lambs were weaned at 17 weeks of age.

2.2. Faecal egg count

Faecal samples were obtained from the rectum of Suffolk ($n = 122$) and Texel ($n = 153$) 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, using the modified McMaster method (MAFF, 1986). Eggs of *Nematodirus* spp. were enumerated separately from those of other *Trichostrongyle* species.

2.3. IFN-γ intron 1 genotyping

IFN-γ intron 1 genotype was determined for 150 Suffolks and 161 Texels. DNA was extracted from muscle using the QIAamp® DNA Mini Kit Tissue protocol (Qiagen, UK) for lambs born in 1999. Blood samples were collected from lambs in flocks born from 2000 to 2002 and DNA extracted using a cold lysis buffer and proteinase K digestion technique.

Approximately 100 ng of genomic 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, MA, USA). A cycle consisted of 1 min denaturation at 94 °C, 1 min annealing at 56 °C and 1 min extension at 72 °C. The forward primer (5′-TGATGTTTGATGTTGACTTG-3′) and reverse primer (5′-GATTGGGTATATATCAT-3′) pair amplified part of intron 1 of the IFN-γ gene, containing both the microsatellite and a SNP located 49 bp downstream of the microsatellite. Primers were designed using the nucleotide sequence of intron 1 of the IFN-γ as a template (GenBank Accession No. Z73273). PCR products containing the large microsatellite were 220 bp in length and those containing the smaller microsatellite were 4 bp shorter.

Initial sequencing of 10 animals from each breed was performed to confirm A and B haplotypes as previously described (Schmidt et al., 1996; GenBank Accession No. Z73273). For sequencing, PCR products were cleaned using Microcon® YM-100 centrifugal filter devices (Millipore, MA, USA) and DNA concentration determined by comparison with a low mass DNA ladder (Invitrogen, Life Technologies, CA, USA). Approximately 8 ng of PCR product were sequenced using the forward primer for intron 1 of the IFN-γ 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., Qld, Australia). IFN-γ intron 1 haplotypes were genotyped by visual analysis of the number of microsatellite repeats and the identity of the SNP on the chromatogram.

Sequencing analysis confirmed the presence of both A and B haplotypes in Texels. However, in Suffolks, two new additional haplotypes were identified, C and D.

As the Texel breed was bi-haplotypic at intron 1 of the IFN-γ gene, the microsatellite was analysed using restriction fragment length polymorphism (RFLP). The 'G' to 'A' SNP 49 bp downstream of the microsatellite in the B haplotype, created a restriction digest site for the restriction enzyme *Bsp*H1, and thus distinguished between the large and small microsatellites. Approximately 8 μl PCR product was incubated with 2.5 Units of the restriction digest enzyme *Bsp*H1 (Biolabs, New England) at 37 °C for 2 h. Restriction digest cut the 216 bp haplotype B into a 174 bp and a 42-bp fragment while haplotype A remained uncut. Resulting band sizes were separated by agarose gel electrophoresis and size determined by comparison to a 50-bp DNA ladder (Invitrogen, Life Technologies, CA, USA). RFLP analysis could not distinguish all haplotypes in Suffolks, consequently IFN-γ intron 1 genotyping in this breed was performed by sequencing as described above.

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