THE IDENTIFICATION OF SHEEP GENETICALLY RESISTANT TO THE NEMATODE *TELADORSAGIA CIRCUMCINCTA*

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INTRODUCTION

Gastrointestinal nematode infection in sheep is a relatively well-understood host-parasite interaction. Usually, the presence of infection can be diagnosed by nematode eggs in the faeces. There is also a need to identify relatively susceptible animals for selective anthelmintic treatment or culling. A variety of indicator traits and genetic markers exist. The purpose of this article is to discuss the advantages and disadvantages of each method, especially in relation to natural, predominantly *Teladorsagia circumcincta* infection.

MATERIALS AND METHODS

Complete descriptions of the methods employed are available: faecal egg counts (Stear *et al.,* 1995), parasite specific IgA activity (Strain *et al.,* 2002), Western blotting (Strain and Stear, 1999), peripheral eosinophilia (Stear *et al.,* 2002), pepsinogen concentration (Stear *et al.,* 1999), plasma albumin concentration (Stear *et al.,* 2000), fructosamine concentration (Stear *et al.,* 2001), *DRB1* typing (Schwaiger *et al.,* 1995) and interferon gamma typing (Coltman *et al.,* 2001).

RESULTS AND DISCUSSION

The methods used to identify relatively resistant and susceptible animals can be divided into indicator traits and genetic markers. The indicator traits can be further subdivided into parasitological, immunological and pathological traits. These will be considered separately.

Parasitological indicator traits. Faecal egg counts provide an unequivocal demonstration of the presence of nematode infection. They are heritable (Bishop *et al.,* 1996) and provide sufficient information to justify selective breeding. However, the relationship between faecal egg count and number of adult worms is convex (Bishop and Stear, 2000) ; animals that are infected with large numbers of *T. circumcincta* produce very few eggs. The absence of eggs in the faeces does not indicate the absence of infection.
In addition, the heritability of faecal egg count varies with age and rises with increasing exposure to nematodes (table 1). Care is needed to avoid working with animals that are too young since in these animals the maternal effect is large but the genetic component is low. Similarly faecal egg counts are unlikely to discriminate relatively resistant from relatively susceptible animals if the flock has not been exposed to long-term infections or if some or all of the animals in the flock have recently been treated with anthelmintic.

Table 1. The heritability of faecal egg count increases with age

Month	Estimate ± standard error
May	0.01
June	0.00
July	0.12 ± 0.10
August	0.14 ± 0.12
September	0.15 ± 0.07
October	0.22 ± 0.13

Less heavily infected animals have fewer adult nematodes, shorter adult nematodes and a greater proportion of early fourth-stage larvae in inhibition. Of these traits only the length of adult nematodes is under strong genetic control (table 2) and there is no easy method of measuring these traits in live animals.

Table 2. Heritability of indicator traits for nematode infection

Trait	Age at measurement	Estimate ± standard error
Number of adult T. circumcincta	6 – 7 months	0.14 ± 0.10
Adult female worm length	6 - 7 months	0.62 ± 0.20
Number of fourth-stage larvae	6 - 7 months	0
Parasite-specific IgA activity	5 months	0.56 ± 0.11
Eosinophil concentration	5 months	0.43 ± 0.17
Fructosamine concentration	5 months	0.34 ± 0.14

Immunological indicator traits. A major source of variation among animals in genetic resistance to nematodes appears to be genetic variation in protective, immunological responses. Therefore, traits based on immune responsiveness are attractive candidates for assessing genetic resistance. However, immunological traits need sustained and prolonged exposure to nematode infection before genetic variation becomes apparent.

Increased IgA activity in the abomasum against fourth-stage larvae is strongly associated with smaller adult female *T. circumcincta* (Stear *et al*., 1995). There is a non-linear relationship between the amount of IgA in the abomasum and in the peripheral circulation (Stear *et al.,* 1995). Parasite specific peripheral IgA activity is highly heritable (table 2) but accounts for only 6 % of the observed variation in adult female worm length.

Western blotting of extracts from fourth stage larvae reveals that certain parasite molecules are preferentially recognised by resistant but not susceptible animals. In particular, sheep that recognise bands of 87,000 Mr and 129,000 Mr in fourth-stage larvae have shorter adult female worms (Strain and Stear, 1999). Similarly, sheep that recognise bands of 28,000 Mr and 37,000 Mr in adult parasite extracts have shorter worms than sheep that fail to recognise these molecules. Nevertheless, more development is needed, especially on the preparation of parasite molecules before Western blotting can be recommended for screening large numbers of animals.

Peripheral eosinophilia and eosinophil related traits have been associated with resistance to *T. circumcincta* (Stevenson *et al.,* 1994 ; Doligalska *et al.,* 1999 ; Stear *et al.,* 2002) and to other

species of nematodes in sheep (Douch *et al.,* 1996). However Woolaston *et al.* (1996) has queried the usefulness of peripheral eosinophilia as a marker of resistance to infection. Differences among studies in the exposure of animals to nematodes may explain inconsistencies among different groups (Douch *et al.,* 1996).

Pathological indicator traits. In addition to markers based on immune responses, markers based on pathological changes have also been studied. Plasma pepsinogen concentrations are influenced by both the number and the mean length of the *T. circumcincta* population. However, as only worm length appears to be under strong genetic control, pepsinogen concentrations are perhaps better at identifying heavily infected rather than resistant animals.
Plasma albumin concentrations fall following infection but albumin concentrations appear less useful markers than fructosamine concentrations (Stear *et al.,* 2000). Among animals grazing the same pasture, fructosamine concentrations reflect relative intensities of infection (Stear *et al.,* 2001). In addition, fructosamine concentrations are moderately heritable (table 2). Variation in fructosamine concentrations is influenced by relative protein status and hence variation in nutrition is also likely to be important (Stear *et al.,* 2000). In addition, animals with relatively low fructosamine concentrations subsequently acquire relatively high worm burdens (Stear *et al*., 2001), possibly because they modify their grazing behaviour (Hutchings *et al.,* 1999).

Genetic markers. Genetic markers are - in theory - free of many of the problems that beset the use of indicator trait such as the need for prolonged and sustained exposure to parasites and the influence of nutrition on pathogenesis and immune responsiveness. However, great care is needed during the gene hunting process. Genes that operate through immune responsiveness will only show significant associations with indicator traits if the immune response is well developed. In other words during the gene hunting process there is a need to ensure prolonged and sustained exposure to nematodes and to minimise variation in past and current nutrition.
There are two loci that have shown associations with parasite resistance in different experiments. One is the major histocompatibility complex, especially the *DRB1*, the other is a region on chromosome 3 encompassing the interferon gamma *(IFNG)* (Crawford and McEwan, 1998). Both *IFNG* and *DRB1* regulate immune responses, although it is not clear if these are the true disease susceptibility loci or merely markers. At low densities of infection variation among animals is dominated by differences in worm burdens and genetic effects are weak. At higher densities of infection, differences in parasite fecundity contribute more to the variation among animals in faecal egg output and genetic effects are stronger. Therefore the magnitude of any allelic effect is likely to vary at different intensities of infection and will therefore be a complicated function of grazing management, pasture contamination, frequency of anthelmintic treatment, weather and relative nutritional status.

CONCLUSION

As with all complex traits, variation among animals in resistance to nematode infection is influenced by both genotype and environment. The big advantage in the case of ostertagiasis is that we have identified many of the genetic and environmental sources of variation. These combine to produce a fascinating interplay that determines susceptibility to disease and

infection. A variety of phenotypic indicator traits and genetic markers exist. However, none of these markers are foolproof. Great care and forethought in their use is required.

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