Selective breeding for the control of nematodiasis in sheep

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Summary: Genetic manipulation of sheep by selective breeding offers a means to reduce the current reliance on chemotherapy for the control of gastro-intestinal nematodes. Simulated epidemiological studies support this view as, compared to lambs of 'normal' susceptibility, those 'selected' for resistance to Trichostrongylus colubriformis have lower worm burdens and reduced production losses. Considerable genetic variation exists both between and within breeds of sheep, and a number of breeding programmes have demonstrated that selection for animals with heightened levels of resistance to nematodes is feasible. Animals from these selection experiments are currently being used to investigate the nature of this genetic regulation and the economic benefits that can be achieved. An understanding of the mechanisms of resistance, facilitated by having animals with defined extremes of responsiveness, is crucial for studies into the specificity of selection, identification of predictive markers with resistance, and determination of suitable vaccines and vaccination strategies in unselected populations. Immunity plays a major role in host resistance to parasites, and from studies with selected animals, it appears that a broad range of immune responses are under genetic control. Genetic diversity within the parasite population may manifest itself in adaptation to withstand host resistance mechanisms. Such an occurrence could limit the effectiveness of the genetic approach.


INTRODUCTION

The widespread occurrence of gastro-intestinal nematodes and the consequent loss of animal production result in a problem of considerable magnitude for the pastoral industries. It has been suggested (9) that parasites can reduce the profitability of sheep production through:

a) mortality, which includes the capital cost of replacement

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b) quantitative and qualitative reductions in liveweight gains, wool growth and reproductive efficiency

c) increased costs of production arising from the use of anthelmintics and the labour involved in their administration

d) opportunity costs foregone by avoiding or spelling pastures known to be highly contaminated.

The total direct and indirect costs resulting from parasitic infections can therefore be substantial. In Australia, the Bureau of Agricultural Economics (13) has attempted to evaluate the economic costs associated with internal parasite infection. In a year of 'normal' prevailing weather conditions the costs to the Australian sheep industry (in 1985 values) were partitioned into A$ 309m of production losses and A$ 53m for the use of drenches and their administration.

The advent of modern broad-spectrum anthelmintics promised an efficient and reliable means of controlling the economically-important gastro-intestinal nematodes. As these drugs were initially highly effective against both immature and adult worms, control practices became virtually reliant upon anthelmintics in either a curative or preventive role (28). However, the increasing incidence of resistance in nematodes to anthelmintics (54), high developmental costs involved in identifying new anthelmintics and stringent government regulations involved in the registration of new drugs (33) limit the scope of future chemical control. In addition, an emerging force is the increasing community awareness of environmental issues, and consumer demand for animal products and pastures free of chemical residues. For these reasons, alternative and/or complementary control options are being sought which reduce the current reliance on chemotherapy.

One control strategy which shows considerable promise is the utilisation of genetic variation within the sheep population to increase flock resistance to nematode species. This paper will examine epidemiological aspects of having flocks of resistant animals, selection experiments in Australia and New Zealand designed to establish lines of sheep having superior resistance principally to either the intestinal nematode *Trichostrongylus colubriformis* or the abomasal nematode *Haemonchus contortus*, and the immunological and parasitological implications arising from these investigations. The emphasis given to the *Trichostrongylus* selection programme in this review reflects the involvement of the author in this research area.

**THE EPIDEMIOLOGICAL IMPACT OF INCREASED FLOCK RESISTANCE**

Barger (10) has carried out a study in which the frequency distributions for four gastro-intestinal nematode species were determined in naturally-infected lambs. He found that in each case a negative binomial distribution occurred which empirically described a situation of overdispersion. In overdispersed distributions, a relatively small proportion of the host population carries a large proportion of the parasite population. It has been stated (46) that the most resistant 50% of the flock produce less than 10% of the worm-eggs counted, whereas the most susceptible 15% of the population produce 50% of the eggs counted. The existence of susceptible host
genotypes and the overdispersed distribution of worm populations may be a means whereby the parasite is able passively to evade resistance mechanisms of the host and survive to the next generation (52). Furthermore, host diversity (which includes both resistant and susceptible genotypes) is conserved, albeit with due consideration to natural selection, and is a means whereby evolutionary fitness is maintained in the presence of the parasite.

Dineen (21, 25) has proposed that immunological control of parasite infections is mediated about thresholds of antigenic information; burdens above the threshold trigger immunological mechanisms whereas those below are subliminally tolerated. Over time, as immunoselection of the parasite is directed towards antigenic identity, or reduced antigenic disparity, with the host, larger worm burdens are required to reach the antigenic threshold. In this situation, antigenic identity would not be achieved because the pathogenic effects of the subliminally tolerated worm burden would limit the fitness of that host genotype. Thus, a dynamic equilibrium exists between host and parasite genotypes, with reduced antigenic disparity and pathogenicity being counterbalanced. Man has interfered with this evolutionary balance by indirectly promoting the survival of a disproporionate number of susceptible animals through the use of modern broad-spectrum anthelmintics (52). Selective breeding for resistant genotypes (= selection against susceptibility) offers the opportunity to decrease the overall susceptibility of a flock.

The epidemiological consequences of having a flock of resistant lambs (due either to selection or vaccination procedures) have been modelled in a versatile computer programme developed by E.H. Barnes and R.J. Dobson at McMaster Laboratory, Sydney (12). This programme is able to predict the dynamics of *T. colubriformis* infection due to host influences on establishment rates, fecundity and rejection of adult worms, and estimate larval availability on pasture as influenced by faecal egg count (FEC) data and standard meteorological information. Barger (11) has used the model to simulate the epidemiological consequences of having flocks of lambs either resistant or with normal susceptibility which are treated or untreated with anthelmintics. In his simulation, genetic resistance was generated by reducing the worm burden (threshold) above which immunological mechanisms act to regulate the infection. This work showed that, compared to drenched lambs of normal susceptibility, undrenched resistant lambs had lower mean worm burdens and resulted in lower larval contamination on pasture.

Similar studies have been carried out using a later version of the model to simulate parasitological data for lambs having ‘normal’ susceptibility or those having been ‘selected’ for increased resistance. In this case, resistance was generated by removing a factor controlling age-related unresponsiveness, as it has been shown (23) that some lambs are as responsive to *T. colubriformis* as mature sheep. Combinations of three parameters were varied in different runs of the programme, namely,

a) weaners either moved to clean pastures at weaning or set stocked on the same paddock with the ewes removed at weaning

b) ewes either remained untreated or were treated with anthelmintic (drenched) prior to lambing and at weaning

c) lambs either remained undrenched or were drenched three times (before weaning, at weaning and three months after weaning).
The drenching regime used in the model follows recommendations developed for and widely adopted in the Armidale region of N.S.W. (19). Each simulation was run using twenty years of actual weather data from the Pastoral Research Laboratory, Armidale, with the arbitrary initial values described by Barger (11). For the model, it is assumed that individual deaths occur when worm burdens exceed 50,000 adults. However, due to the negative binomial distribution, deaths within a flock commence at a mean worm burden of 25,000 as at this level some individuals have worm burdens over 50,000 adults. It is also assumed that production losses commence at adult worm burdens of 7,500. These limits, indicated on Fig. 1, are based on extensive experience of parasitological data obtained at the Pastoral Research Laboratory.

Table I shows the percentage of deaths, means of the peak adult *T. colubriformis* worm burdens, and means of the peak larval contamination on pasture for lambs during the period from birth to twelve months of age over the twenty simulated years. In each case, these values are lower for 'selected' (resistant) lambs than for 'unselected' lambs having normal susceptibility. As would be expected, differences between the two lamb categories are smallest under the most intensive management conditions (Treatment 1). In this case, 'selected' lambs had a 38% reduction in mean peak worm burden and a 53% reduction in mean peak larval contamination on pasture. The maximum differences between lamb categories occurred when weaners were set stocked and both ewes and lambs were drenched (Treatment 3). In this case, 60% mortalities occurred in 'unselected' lambs whereas no deaths occurred in 'selected' animals. Furthermore, 'selected' lambs had a 78% reduction in worm burden and resulted in an 85% reduction in pasture contamination. A gradual increase in worm burdens and a corresponding rise in lamb deaths occurred for the various drenching regimes in set stocked lambs (Treatments 3 to 6). Where virtually no control measures were employed (Treatment 6), 'selected' lambs still had a 56% and 74% reduction in worm burden and larval contamination, respectively. However, in contrast to the 82% mortality in 'unselected' lambs, 'selected' animals had only 13% deaths.

Figure 1 shows the peak adult *T. colubriformis* worm burdens and peak larval contamination on pasture in each year for Treatment 3, where maximal differences between lamb categories occurred. In addition to the differences already described, severe production losses occurred in each year for 'unselected' lambs whereas in 'selected' animals mean worm burdens exceeded those required to lower production in 11 of the 20 years.

The results described here agree with those of Barger (11) who concluded that it would not be necessary to drench genetically resistant sheep at anywhere near the same frequency required by unselected animals. In addition to increased production, reduced costs involved in anthelmintic treatment and decreased selection pressure on the parasite for anthelmintic resistance, the use of host genotypes resistant to parasites led to lower larval numbers on pasture and decreased exposure for the flock as a whole. The necessity to avoid or spell pastures known to be highly contaminated would then be lessened. Thus, in general terms, the model demonstrates the practical advantages that can be achieved by having flocks of resistant animals.

**GENETIC VARIATION IN RESISTANCE TO INTERNAL PARASITES**

Evidence for genetic variation in sheep for resistance comes from both between- and within-breed comparisons. These studies, which relate mainly to *H. contortus,*
### TABLE I

**Results of computer-simulated lamb mortality, mean peak adult T. colubriformis worm burdens, and mean peak larval contamination on pasture during the period from birth to 12 months of age**

Various control options are modelled in flocks of 'unselected' (normal susceptibility) and 'selected' (resistant) lambs over a twenty year period.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lamb class</th>
<th>Lamb deaths %</th>
<th>Mean peak adult worm burden</th>
<th>Mean peak larvae on pasture L₃/kg DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Weaners moved/ewes drenched/lambs drenched</td>
<td>Unselected</td>
<td>0</td>
<td>8,632</td>
<td>3,844</td>
</tr>
<tr>
<td></td>
<td>Selected</td>
<td>0</td>
<td>5,319</td>
<td>1,822</td>
</tr>
<tr>
<td>2. Weaners moved/ewes undrenched/lambs undrenched</td>
<td>Unselected</td>
<td>4</td>
<td>18,595</td>
<td>26,664</td>
</tr>
<tr>
<td></td>
<td>Selected</td>
<td>0</td>
<td>10,474</td>
<td>10,623</td>
</tr>
<tr>
<td>3. Weaners set stocked/ewes drenched/lambs drenched</td>
<td>Unselected</td>
<td>60</td>
<td>44,238</td>
<td>48,433</td>
</tr>
<tr>
<td></td>
<td>Selected</td>
<td>0</td>
<td>9,679</td>
<td>7,274</td>
</tr>
<tr>
<td>4. Weaners set stocked/ewes undrenched/lambs drenched</td>
<td>Unselected</td>
<td>63</td>
<td>46,292</td>
<td>41,837</td>
</tr>
<tr>
<td></td>
<td>Selected</td>
<td>2</td>
<td>13,651</td>
<td>8,851</td>
</tr>
<tr>
<td>5. Weaners set stocked/ewes drenched/lambs undrenched</td>
<td>Unselected</td>
<td>80</td>
<td>51,874</td>
<td>57,031</td>
</tr>
<tr>
<td></td>
<td>Selected</td>
<td>8</td>
<td>24,832</td>
<td>14,193</td>
</tr>
<tr>
<td>6. Weaners set stocked/ewes undrenched/lambs undrenched</td>
<td>Unselected</td>
<td>82</td>
<td>54,744</td>
<td>57,169</td>
</tr>
<tr>
<td></td>
<td>Selected</td>
<td>13</td>
<td>24,073</td>
<td>14,822</td>
</tr>
</tbody>
</table>

have been the subject of a number of recent reviews (11, 16, 31, 42) and therefore will not be considered in detail here. It is pertinent to note, however, that in a comparison of published reports for *H. contortus*, variability within breeds for resistance may be as great as the variability between breeds (31). This finding supports the view (36) that sufficient genetic variation exists within the Australian Merino population to make a programme of selective breeding feasible. Although there is relatively little information available documenting between- and within-breed resistance to *Trichostrongylus* spp., there is no reason to suggest that different conclusions should be drawn.

### SELECTION FOR RESISTANCE TO GASTRO-INTESTINAL PARASITES

Although empirical descriptions of genetic variability in responsiveness to nematodes have existed for some time, it has only been within the last twenty years...
A. MODELLLED PEAK WORM BURDENS

B. MODELLLED PEAK LARVAL CONTAMINATION ON PASTURE

**Fig. 1**

Computer-simulated parasitological data pertaining to *T. colubriformis* for lambs which are either 'unselected' (that is, having normal susceptibility) or 'selected' (resistant) whilst grazing pasture

Management procedures involved set stocking lambs on the one pasture and drenching both ewes and lambs (Treatment 3 – see text for details).

**A.** The peak adult *T. colubriformis* worm burdens for each year in 'unselected' and 'selected' lambs. Estimates of the worm burdens where production losses commence, deaths within the flocks commence, and individual deaths occur are indicated.

**B.** The peak larval contamination on pasture (L3/kg dry matter) in each year resulting from grazing by 'unselected' or 'selected' lambs.
that intensive studies into this genetic control have been initiated. These programmes aim to utilise and manipulate within-breed variability in the host genome by selection. A number of ovine breeding programmes are currently underway in Australia and New Zealand and have broadly similar objectives, although each has originated from diversified beginnings in terms of testing procedures and the parasite species used. These objectives are to:

- characterise the nature of the genetic regulation of resistance
- understand the underlying mechanisms involved in resistance
- estimate genetic correlations between parasite resistance and economically important traits (for example, wool growth, liveweight gain and fertility)
- determine the specificity of selection in terms of other parasites and non-parasitic pathogens
- identify predictive markers with resistance.

A brief description of these selection programmes follows.

**Trichostrongylus selection lines**

In a breeding programme initiated at McMaster Laboratory, selection and assortative matings have produced lines of Merino sheep in which lambs are either responsive (high responders) or susceptible (low responders) to homologous challenge after vaccination with irradiated *T. colubriformis* larvae (56, 57). A primary aim of this work has therefore been to focus on the genetic control of immunological responsiveness to gastro-intestinal nematodes in lambs. All animals, apart from those in field trials, were assessed at a young age under controlled conditions in pens so as to standardise environmental influences and allow assessment of immunological reactivity to defined parasite burdens. Individual responsiveness has been based on FEC and estimated by the mean number of worm-eggs per gram of faeces (epg) collected on five occasions after challenge. A random (unselected) line provided animals of each sex which were either vaccinated (controls for selection) or unvaccinated (parasitological controls). Selection has now proceeded into the fourth of arbitrarily designated generations. For each generation, lambs from the high responder line had significantly lower FEC than low responders, and, in addition, within each line, female lambs were more immunologically responsive than males. Figure 2 shows the response to selection to the end of the fourth generation, and is expressed in terms of protection which relates the FEC of selected groups with those of sex-matched unvaccinated randoms. For the high responder line, and particularly the females, a large response to selection has occurred in the first generation with more modest increases occurring subsequently. The relatively poor response to selection for the later generations in the low responder line may be due to a limit imposed by the relatively moderate challenge dose. Heritability for the response to vaccination (± standard error) was estimated to be 0.41 ± 0.19, and this corresponded closely to the realised heritability of 0.39 ± 0.27 (56). Although this work uses vaccination as a means of generating acquired responses in a labour efficient manner, prior sensitisation is not necessary to demonstrate differences between the high and low responder lines. In contrast to low responders, unvaccinated high responders are able to mount a vigorous immune response to challenge after a brief initial period of high FEC (57).
Response to selection in the *Trichostrongylus* high responder (HR) and low responder (LR) lines expressed as the level of protection after vaccination and challenge with *T. colubriformis* larvae in five generations (parent, G1, G2, G3, G4).

Protection was calculated by comparing FEC of vaccinated groups with those of sex-matched unvaccinated random lambs in each generation.

*Haemonchus* selection lines

At the Pastoral Research Laboratory, “Chiswick”, Armidale, N.S.W., divergent lines have been established with either increased or decreased resistance to *H. contortus* (42, 58). In this work, selection has been based on the maximum FEC following artificial infection given to 5-6 month old Merino lambs while on pasture. A randomly-bred unselected line provides relevant controls for the selection. Little response to selection occurred in the early years; however, from the fifth year there were consistent significant differences between lines. Heritability for resistance to *H. contortus* in this experiment was estimated to be 0.33 ± 0.03.

**UNE H. contortus** resistance flock

Initially, work based at the University of New England (UNE), Armidale, was designed to estimate the degree of genetic variability within the Merino population for resilience and resistance to *H. contortus*. These parameters were measured in the...
progeny of 61 sires following artificial infection while on pasture (3, 4, 5). Resilience, defined as the ability to maintain production during infection, was found to have a low heritability which did not differ significantly from zero. In contrast, heritability for resistance, the ability to suppress establishment and/or subsequent development of the infection, was 0.34 ± 0.10. The identification of a ram, whose progeny exhibited extreme resistance, has provided the impetus to carry out an intensive examination of this trait. Matings are being carried out between relatives of this ram in order to verify the hypothesis concerning the involvement of a major gene and to determine the underlying mechanisms.

Selection programmes based on naturally-acquired field infections

Two selection programmes have been reported which are directed against naturally-acquired field infection consisting predominantly of *Trichostrongylus* and *Ostertagia* spp. In New Zealand, divergent lines of Romney sheep have been established on the basis of FEC during two sampling periods separated by anthelmintic treatment, the first commencing at five months of age (8). Response to selection has been greatest when based on FEC in the second period, and probably reflects the involvement of genetic regulation of the acquired resistance. Other workers in Australia (46, 48) are using lymphocyte responsiveness to parasite antigen, together with FEC data, to breed Merino lambs resistant to pasture infection. Detailed results from this selection experiment have yet to be reported.

A common feature of the selection programmes described is the lack of a negative association between resistance and production parameters (5, 56, 58). On the basis of genetic correlations between FEC and liveweight gain in the UNE resistance flock, it was concluded that selection for resistance would not influence production in worm-free animals, but during infection lambs selected for superior resistance would have increased production (5). A recent report (58) indicates that fertility may be adversely affected by selection towards susceptibility.

The heritabilities for resistance to infection are remarkably consistent (0.3-0.4) and results from the selection programmes suggest that relatively rapid genetic gains can be achieved. The manner in which selection for resistance to parasites could be included in commercial breeding programmes depends on a number of factors which as yet are poorly defined (4, 43, 58). By comparing predicted responses to four different breeding strategies, it has been shown that progress will be most rapid when selection is based on a major gene (4). However, even in the absence of a major gene, it has been estimated (43) that income per ewe per year could be increased in a moderately parasitised environment by 10% annually by including parasite resistance in a selection index from which superior animals are chosen.

MECHANISMS OF RESISTANCE

The importance of immune responses in the intra-host regulation of parasite burdens is seen most clearly when this control is suppressed (for example, in the lactating, nutritionally-deprived or stressed) or not fully developed (as in the neonate) (21). It appears that genetic influences may determine the extent of susceptibility within these categories. Thus, strain and breed differences existing in the expression of the
periparturient rise in FEC is due to genetic variation in the relaxation of immunity (18). The rise in FEC during the periparturient period is an important epidemiological consideration as pasture contamination is increased at a time when lambs, which as a whole are relatively more susceptible than mature animals, begin to graze. This age-related unresponsiveness, however, has also been shown to be under genetic control (23). Vaccination with irradiated *T. colubriformis* larvae therefore resulted in a segregation of lambs into those categorised as ‘responders’ (having comparable levels of protection to mature animals) or ‘non-responders’ (having worm burdens similar to unvaccinated controls). Because groups challenged as lambs or mature animals were vaccinated at the same time (12 and 14 weeks of age), it appears that lambs can be ‘primed’ but some are unable to mount protective responses until the effector arm of immune responsiveness matures at a later age. This variability in young lambs was thought to be genetically controlled, and the *Trichostrongylus* selection flocks were initiated to investigate the nature of this regulation of acquired resistance. This selection is therefore overtly immunologically based. The superior resistance to *H. contortus* of progeny within the UNE resistance flock following infection (5) also has an immunological component as treatment with the immunosuppressive agent dexamethasone abolished differences between resistant and susceptible animals (44).

The immune response to parasites consists of a number of complex interactions, and in laboratory host/parasite models a number of immunological functions are under genetic control (53). Mechanistic studies in each of the sheep selection programmes are still in the early stages, with much of this work to date being limited to the assessment of peripheral reactions as the animals have been required for breeding. It is expected that this research will gain increasing momentum as the flocks expand and animals surplus to breeding requirements become available for detailed investigation of responses at the site of infection.

In the *Trichostrongylus* selection flocks, a number of studies have been carried out to assess the immunological competence of progeny from the high and low responder lines. Results to date suggest that selection for a broadly based character such as response to vaccination has influenced responses in a wide range of immunological functions. As would be expected, high responders have performed better than low responders. Aspects of cellular and humoral antigen recognition were improved in high responders. Thus, the *in vitro* blastogenic responses of antigen-reactive lymphocytes (26, 55) and antibody levels to parasite antigen, as measured by the complement fixation test (35) or ELISA (Windon, unpublished), were greater in high responders following vaccination and challenge. These animals also had higher levels of the complement component C3, but not C4, after infection (32). C3 may play a role in the activation, via membrane receptors, of a number of cell types including those involved in phagocytosis and mediator release (29). Peripheral blood leukocytes of high responder lambs had increased phagocytic activity, as measured by chemiluminescence (56). This assay may also indirectly measure the increased capacity of high responders to produce a number of non-specific mediators (such as prostaglandins, leukotrienes and thromboxanes) and stimulate the release of amines from mast cells (see 56). An examination of the release of pharmacologically-active mediators (35) showed that during infection high responders had elevated levels of histamine in intestinal tissue, and higher concentrations of leukotriene B4 and leukotriene C4 in duodenal mucus but not tissue. Of the cells implicated in release of these mediators, globule leukocytes (thought to be degranulated mast cells)
were found in greater abundance in the epithelium of the small intestine of resistant progeny (26), and high responders had elevated numbers of circulating eosinophils during infection (20).

There is evidence to suggest that the immune response influences qualitative as well as quantitative aspects of the worm burden. The various manifestations of resistance include the failure of infective larvae to establish, inhibition of development in larval and adult worms, decreased egg production by females, expulsion of adult worms, and discrimination against one sex of the parasite (21). Responsive wethers from the *Trichostrongylus* selection flocks had consistent positive correlations between parameters measuring the magnitude of the effect of resistance on the parasite (worm length, eggs *in utero* and sex ratio) and the vigour of the response to challenge (worm counts) (27). In contrast, the corresponding correlations for susceptible lambs and unvaccinated controls tended to be negative, perhaps due to density-dependent functions.

The existence of lines of sheep possessing defined responsiveness to nematodes provides an ideal framework for efficiently and effectively studying mechanisms under genetic regulation. Such studies have important consequences for the identification of potential predictive markers with resistance, and for the development of vaccines and vaccination strategies which aim to correct the defect existing in low responder genotypes by immune manipulation. Vaccination may produce only the levels of protection seen in the field (21) and the genetic regulation of responsiveness may therefore be a major constraint on the success of any vaccine.

**SPECIFICITY OF SELECTION**

Evidence exists from experiments carried out with laboratory host/parasite models that selection for responsiveness to non-parasite antigens and parasites can be non-specific. In the high and low antibody lines of mice developed by Biozzi and his colleagues (14), interline differences were determined by macrophage catabolism of antigen. Thus, in the low line, accelerated macrophage activity removed antigen before the necessary cellular interactions occurred for synthesis of antibody, whereas persistence of antigen in the high line stimulated antibody-producing cells. Observed responses to unrelated antigens (parasite and non-parasite) reflected the limiting immunological mechanism. For instance, if antibodies play a dominant role in host defence against a particular pathogen, the high antibody line is advantaged, but on the other hand, if macrophage activity is important, the low antibody line is advantaged. In a manner remarkably similar to the Biozzi mice, lines of mice selected for response to single (15) or multiple (50) infections with *Nematospiroides dubius* also show that some degree of specificity exists which could involve limiting mechanisms. Such results have led to the hypothesis that selection for resistance to one organism may focus on a particular immune function and perturb an immunological equilibrium, thereby predisposing susceptibility to other pathogens (22). In this regard, it is of interest to note that preliminary data from the *Trichostrongylus* selection lines has shown that antibody responses to the synthetic antigens DNP.BSA and TNP.LPS are higher in low responder lambs (56).

Grazing sheep usually encounter a number of parasitic species on pasture. Thus, the practical importance of selection programmes to graziers will be enhanced if a
general increase in resistance status occurs for a range of gastro-intestinal species and, indeed, greater overall immunological competence. Cross-resistance between generically unrelated intestinal nematodes in sheep has been shown to involve components which are immunologically specific (those involving antigen recognition and triggering inflammatory reactions) and non-specific (the end products of the inflammatory response) (24). Thus, vaccination with one species did not confer protection to heterologous challenge unless the homologous species (the ‘trigger’) was also present. In addition to this, it would be expected that animals selected for resistance to one species will also demonstrate protection against heterologous species if the mechanisms involved play an equally important role for both.

Some evidence is available on the specificity of selection to other nematodes from the selection programmes. Lambs from the *T. colubriformis* selection flocks have been tested for responsiveness vaccination and challenge with *H. contortus* in pens after the initial *T. colubriformis* testing. On the basis of FEC after challenge, no differences were observed between high and low responders in second generation lambs (56). A closer examination of responses to *H. contortus* was then carried out using third generation progeny (57). As opposed to the high levels of protection achieved against *T. colubriformis* in high responders (79% and 88% for males and females, respectively), only moderate protection occurred against *H. contortus* (30% and 51% for males and females, respectively). Significant positive correlations between FEC from each infection indicated that individual resistance was consistently expressed between species. Similarly, with the Chiswick *Haemonchus* flocks, that selection for resistance to *H. contortus* is only partially effective against *T. colubriformis*. Thus, the difference observed between the increased resistance and decreased resistance lines for artificial infection with *T. colubriformis* was 64% of the difference following artificial infection with *H. contortus* (58). However, other workers have found that pastured Romney lambs selected for high or low FEC attributable to *Trichostrongylus* and *Ostertagia* spp. were equally susceptible to *H. contortus* (8).

In addition to *H. contortus*, pen and field studies using animals from the *Trichostrongylus* flocks demonstrated that high responders are more responsive than low responders and random unvaccinates to a number of related and unrelated nematode species (56, 57). These include artificial infection in pens with *Trichostrongylus rugatus*, *Trichostrongylus axei* and *Ostertagia circumcincta*. In addition, results obtained using an isolate of *T. colubriformis* resistant to the anthelmintic levamisole (56) suggest that selection for anthelmintic resistance has not altered the susceptibility of the parasite to immunological attack. In the field, where the predominant species were levamisole-resistant *Trichostrongylus* spp. and *Ostertagia* spp., high responders had significantly lower FEC than low responders or randoms. Additional evidence for cross-protection comes from a field experiment using progeny from the UNE resistance flock or lambs of normal susceptibility to *H. contortus* (G.D. Gray and I.A. Barger, personal communication). FEC, attributable mainly to *Trichostrongylus* spp. with some *Ostertagia* spp., were 75 to 58% lower in resistant progeny compared to susceptible progeny.

In general, it appears that selection for improved responsiveness against one species also leads to greater responsiveness against other nematodes. However, the degree of cross-protection, particularly between the blood-sucking abomasal *H. contortus* and *T. colubriformis* which resides in the intestine and probably feeds by browsing intestinal secretions, may depend on the nature of the parasitism (reflecting differences in evasion strategies employed by each parasite species) and/or the mechanisms
required for immunological control. In contrast to infection with *T. colubriformis*, circulating eosinophil numbers did not correlate with *H. contortus* FEC in lambs from the *Trichostrongylus* selection lines (Windon, unpublished). This could suggest differences in the effectiveness of mechanisms involved in resistance to *T. colubriformis* and *H. contortus*. Further work is required to define precisely the nature and degree of specificity to other gastro-intestinal nematodes in each of the selection programmes. In addition, this needs to be extended to other parasites (for example, cestodes, trematodes and external parasites) as well as to non-parasite pathogens.

**MARKERS WITH RESISTANCE**

The significance to and acceptance by commercial stud breeders and graziers of resistance to parasites as a desirable trait is contingent upon the identification of a predictive marker associated with resistance. Such a parameter should be linked genetically to the trait and therefore could be incorporated into an index of individual performance from which superior animals are selected. A suitable predictive marker would be one which does not require infection with the parasite and is not influenced by environmental or physiological factors that may produce transient effects. The search for predictive correlates is inextricably linked to the underlying basis of the selected trait and highlights the necessity for understanding the mechanisms of resistance. Thus, immunological or physiological parameters which have a genetic basis are common areas for investigation. A number of these have been examined for associations with resistance, with those receiving most attention being discussed below.

**Haemoglobin (Hb) type**

Evans, Blunt and Southcott (30) first reported that sheep Hb type influenced *H. contortus* infections, with animals of the HbAA type having lower faecal egg counts than those with either HbAB or HbBB. While some workers confirmed these findings for *H. contortus* (6, 34, 45) and the non-blood sucking parasite *O. circumcincta* (7), others have been unable to demonstrate associations for *H. contortus* (3, 38, 47), *T. colubriformis* (56) or mixed field infections of *Trichostrongylus* and *Ostertagia* spp. (48).

**Lymphocyte blastogenic responses**

Riffkin and Dobson (47) reported that the preinfection proliferation of sheep lymphocytes to *H. contortus* antigen was inversely correlated with subsequent resistance. The work was extended to infection with *Trichostrongylus* and *Ostertagia* spp. (46, 48) where a significant relationship was reported between the blastogenic responses in six-month-old worm-free lambs and resistance after dosing with "30,000 trichostrongylid larvae". However, work carried out using lambs from the *Trichostrongylus* selection lines was unable to confirm that preinfection blastogenic responses in six- to eight-week-old lambs was a reliable indicator of subsequent resistance (55).

**Ovine lymphocyte antigens (OLA)**

Outteridge and his colleagues at McMaster Laboratory have carried out a series of studies designed to evaluate the association between class I lymphocyte antigen allotypes, thought to be coded for by genes within or closely linked to the Major
Histocompatibility Complex (MHC), and acquired resistance to *T. colubriformis* (39, 41). A panel of typing sera was established and used to identify OLA determinants within the high responder, low responder and random lines. Two antigens of interest were identified: SY1 was found to have increased frequency in the high responder line and decreased frequency in the low responder line, whereas the opposite occurred for SY2. SY1 was subsequently divided into two forms, SY1a and SY1b, which were thought to be alleles at the same locus. Animals possessing SY1a + 1b, pooled over high and low responder lines, had significantly lower FEC than animals with other SY types. Finally, an experiment was conducted to confirm the association and demonstrate that the relationship was not due to a chance foundation effect that occurred within the selection lines (40). Matings were carried out on the basis of SY types in animals unrelated to the selected lines. In a testing regime identical to that of the selected animals, FEC for progeny having SY1a and/or SY1b were significantly lower than those having other types, and therefore confirmed the previous associations. Attempts are being made to further refine the precision of the OLA typing by calculating an index incorporating both SY type and numbers of circulating eosinophils (P.M. Outteridge, personal communication) as evidence from laboratory models suggests that the MHC exerts some influence on eosinophilia (49) and lambs from the high responder line had greater numbers of circulating eosinophils than low responders (20).

Other workers have also examined associations between class I antigens and resistance to other parasite species. Riffkin and Yong (48) found no association between FEC on pasture (thought to be due primarily to *Trichostrongylus* and *Ostertagia* spp.) and SY type in a small group of 39 ewes designated either resistant or susceptible. Investigations into associations between class I OLA type and resistance to *H. contortus* are somewhat contradictory. No relationship was found using both SY and other typing reagents in resistant and control lambs from the UNE flocks (G.D. Gray, personal communication). Similarly, no association was found between OLA type and resistance in lambs from the *Haemonchus* selection flocks (17). However, Luffau *et al.* (38) cautiously state that “association with OLA genotype was not excluded” on the basis of their results and suggest that OLA markers could be valuable indicators of resistance in sheep.

**Restriction fragment length polymorphisms (RFLP)**

One of the uses to which the techniques of molecular biology can be directed is the linkage analysis between DNA markers and segregating characters, in this case resistance to parasites. Restriction enzymes are employed to produce DNA fragments which are then probed in Southern blots (51) with marker genes suspected of influencing a particular trait. Preliminary data from work carried out at McMaster Laboratory by D.J. Hulme (personal communication) using animals from the *Trichostrongylus* high responder, low responder and random lines of sheep have provided encouragement for the success of this approach. In these studies, a human MHC Class II probe identified a particular band which was associated with susceptibility to *T. colubriformis* at a young age. Thus, 34 of the 160 animals tested in which this band was detected had a mean FEC significantly higher than those lambs where the band was not present. This effect was apparent whether data was pooled (after appropriate correction to remove variances attributable to mating type, sire group, sex and experiment year) or restricted to the progeny within each of the selected lines, and accounts for 6% of the phenotypic variance in FEC. Further work is planned.
to confirm and extend these findings by using larger group sizes, different restriction enzymes and other MHC probes. Additional 'candidate' genes for use as probes could be identified by the studies into the mechanisms of resistance.

PARASITE ADAPTATION TO HOST RESPONSES

The discussion so far has concentrated on genetic variability within the host population and its application for the advantage of man. However, the variability that also exists within the parasite population is equally important in the host/parasite interaction. Furthermore, it is possible that artificial breeding programmes based on host resistance would impose a selection pressure on the parasite to favour those genotypes capable of withstanding immunological attack. The development of anthelmintic resistance has provided evidence of the ability of the parasite to adapt genetically so as to ensure its survival. Le Jambre (37) considers that there are parallels in the development of anthelmintic resistance and adaptation of the parasite to resistant hosts. In the case of anthelmintic resistance, selection pressure on the parasite results from a small proportion of exposed genotypes being able to withstand the single mode of action of the chemical. Then, with frequent exposure due to drenching and the reproductive potential of the parasite (large numbers of progeny and short generation intervals), an increase in resistance can be rapid. In contrast, it is considered that host resistance, operating through a variety of mechanisms and probably under polygenic control, would not impose a comparable selection pressure (3). However, if a major gene is involved in resistance, parasite adaptation could occur more rapidly.

Two recent studies in sheep have investigated the ability of *H. contortus* to adapt to host immune responses. In the first (2), unselected animals were either allowed to become immune or remained susceptible because of treatment with dexamethasone. Repeated infections were given to both groups, the last infection providing infective larvae for the next within treatments. After six passages in the untreated animals and nine in the treated animals, groups of susceptible sheep were then infected with larvae from either the immune or susceptible donors. As the resulting FEC showed no differences, it was argued that, in contrast to development of anthelmintic resistance, the equivalent of three years of selection produced no change in the *H. contortus* population. Similarly, other work (1), using five serial passages, showed that *H. contortus* was unable to adapt to host-acquired responses.

Work with the *Trichostrongylus* selection lines has also addressed the question of parasite adaptation (Windon, unpublished). The strain of *T. colubriformis* larvae used routinely for vaccination and challenge throughout the selection programme has been passaged at McMaster Laboratory since 1966 in susceptible lambs. During the challenge period of fourth generation progeny, faeces from fourteen high responder rams having the lowest, but not zero, FEC and five low responder rams having the highest FEC were cultured, and the resulting larvae harvested. The mean FEC of these donors were 253 epg for high responders and 4,453 epg for low responders. Groups of six eight-month-old Border Leicester/Merino ewe lambs, chosen for the vigour of acquired responses compared to pure Merinos, were either vaccinated twice with 20,000 irradiated McMaster *T. colubriformis* or left unvaccinated. Vaccinated and unvaccinated animals were drenched and then challenged with 20,000 larvae cultured from either McMaster (as the unselected
population), high responder or low responder donors. Based on FEC, vaccinated groups challenged with McMaster and low responder larvae had protection of 99.8% and 99.4%, respectively. The group challenged with larvae from high responders had 94.3% protection. For these animals, the mean FEC (± SEM) of 181 ± 27 epg was significantly higher (p < 0.001) than the other vaccinated groups. The level of worm-egg output observed from the high responder worms is such that the potential exists for substantial larval contamination to occur on pasture by adapted parasites. These results are surprising as only one passage occurred through selected hosts, and, in addition, the McMaster strain of *T. colubriformis* may have lost some of the heterogeneity that exists in a wild-type strain. It is planned to confirm and extend the current findings by studying responses to further intensive selection within the parasite population. Work is also underway to examine adaptation of *T. colubriformis* and *H. contortus* to animals from the increased resistance and decreased resistance lines of the Chiswick *H. contortus* selection experiment (58).

**CONCLUSIONS**

The genetic approach is a potentially valuable alternative to the current reliance upon anthelmintics for the control of gastro-intestinal nematodes. However, for selection programmes to contribute worthwhile benefits, a number of criteria should be met. Such programmes therefore need to:

a) be cost-effective in relation to other control measures

b) have reasonable heritability levels to ensure that response to selection will be maximised

c) be non-specific in nature, thereby promoting competence against other nematode species encountered in the field without predisposing for susceptibility to non-parasite pathogens

d) have no detrimental influences on production

e) be based on a correlated character which does not require infection with the parasite to identify resistant animals

f) demonstrate that parasite adaptation to withstand host resistance mechanisms will remain within manageable levels.

Although research using experimental flocks selected for resistance to nematodes has addressed most of these issues, considerable work remains to be done. Once this information is available, appropriate recommendations can then be made as to the most suitable manner for incorporating selection for resistance into commercial breeding enterprises.

It is not envisaged that the end result of this research would totally eliminate the current control procedures (anthelmintic treatment and pasture management). In effect, an integrated approach using conventional practices and genetically resistant sheep would limit production losses and minimise the costs of control. In such a system, the effective life of anthelmintics would be prolonged due to reduced selection pressure on the parasite for anthelmintic resistance. Conversely, the use of anthelmintics at strategic times would retain the advantage of particular host genotypes.
The existence of animals possessing extremes of resistance provides a powerful research tool for studying the mechanisms of resistance to nematodes. Understanding these mechanisms (the basis of the selected trait in breeding programmes) is essential for identifying predictive markers with resistance. In addition, recognition of the limiting mechanisms responsible for susceptibility will allow the opportunity for immunological manipulations to induce resistance. Although vaccination against nematodes with 'non-living' antigens has to date met with only limited success, considerable effort is being devoted to the production of recombinant DNA vaccines. Cellular and antibody probes from animals having extremes of resistance can be used to identify protective antigens upon which these vaccines can be based. However, a vaccine must be effective in genetically susceptible animals and in animals where the immune response is compromised. Genetic constitution may therefore be a major constraint on the success of any vaccination strategy. This is the challenge for immunoparasitologists.

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Résumé: La manipulation génétique obtenue par la sélection des ovins peut constituer une alternative à la chimiothérapie pour lutter contre les nématodes gastro-intestinaux. Cette possibilité est confirmée par des simulations d'études épidémiologiques qui montrent que la charge parasitaire est moins forte chez les agneaux sélectionnés pour leur résistance à l'infestation à T. colubriformis, que chez ceux ayant une sensibilité « normale », et que leur productivité est meilleure. Des variations génétiques considérables existent, tant entre les différentes races qu'au sein de chacune d'entre elles, et un certain nombre de programmes d'élevage montrent qu'il est possible de sélectionner des animaux dont la résistance à l'infestation est élevée. Les animaux ainsi sélectionnés font actuellement l'objet d'études concernant la nature génétique de cette résistance et les avantages économiques qui en découlent. Une bonne compréhension des mécanismes de résistance, facilitée lorsqu'il est possible d'étudier des animaux aux réponses immunitaires extrêmes, est indispensable pour étudier la spécificité de la sélection, identifier les marqueurs de la résistance, et déterminer les vaccins ainsi que les stratégies de vaccination appropriés pour les populations non sélectionnées. L'immunité joue un rôle capital dans la résistance de l'hôte, et il semble qu'un grand nombre de réponses immunitaires soient sous contrôle génétique. La variabilité génétique au sein des populations de parasites peut
LA SELECCIÓN, MEDIO DE PROFILAXIS DE LA NEMATODIASIS DE LOS OVINOS.
– R.G. Windon.

Resumen: La manipulación genética a partir de la selección de ovinos puede ser una alternativa a la quimioterapia en la lucha contra los nematodos gastrointestinales. Esta posibilidad ha sido confirmada por simulaciones de estudios epidemiológicos que muestran que la carga parasitaria en los corderos seleccionados por su resistencia a la infección por Trichostrongylus colubriformis es menos intensa que en aquellos de sensibilidad «normal», y mayor su productividad. Existen considerables variaciones genéticas, tanto entre las diferentes razas como en cada una de ellas, y varios programas de cría muestran que es posible seleccionar animales muy resistentes a la infección. Estos animales son actualmente objeto de estudios tanto respecto de la naturaleza genética de su resistencia como de las ventajas económicas resultantes. Para poder estudiar la especificidad de la selección, identificar los marcadores de resistencia y determinar las vacunas y las estrategias de vacunación apropiadas para las poblaciones no seleccionadas es indispensable llegar a comprender bien los mecanismos de resistencia, lo cual se ve facilitado cuando se pueden estudiar animales con respuestas inmunitarias extremas. La inmunidad desempeña un papel central en la resistencia del huésped, y parece que gran cantidad de respuestas inmunitarias están bajo control genético. La diversidad genética dentro de las poblaciones de parasitos puede manifestarse para adaptar sus reacciones a los mecanismos de defensa del huésped; tal fenómeno podría disminuir la eficacia de la alternativa genética.


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