Investigating genetic aspects of the variation in the host response to gastrointestinal parasites in sheep

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Declaration

I declare that the work presented in this thesis is my own. Specific contributions of others are acknowledged

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List of Publications

Genetic relationships between indicator traits and nematode parasite infection levels in 6 month old lambs. G. Davies, M.J. Stear and S.C. Bishop. Animal Science. 2005. 80; 143 – 150

Genetic relationships between indicator traits and parasitic nematode infection in sheep. G. Davies, M.J. Stear and S.C. Bishop. Proc. of the British Society of Animal Science 2004.

Genetic relationships between indicator traits and parasitic nematode infection in sheep. G. Davies, M.J. Stear and S.C. Bishop. EAAP Book of abstracts No. 10 (2004) Bled, Slovenia.

Quantitative Trait Loci Associated with Parasitic Infection in Sheep. G. Davies, M.J. Stear and S.C. Bishop. Proc. of the British Society of Animal Science 2005.

Use of QTL to Determine Parasite Resistance in Sheep. N. Cockett, S. Bishop, G. Davies, T. Hadfield, S. Eng and J. Miller. Proc. of Joint Annual Meeting ASAS, ADSA and CSAS 2005.

Quantitative Trait Loci Associated with Parasitic Infection in Scottish Blackface Sheep. G. Davies, M.J. Stear, M. Benothman, O. Abuagob, A. Kerr, S. Mitchell and S.C. Bishop. Heredity 96:3 252-258.

Abstract

Gastrointestinal parasites infect all grazing livestock and are a major cause of economic loss. Each year in the UK, gastrointestinal parasites cost the sheep industry an estimated £85 million. Current control strategies are based on anthelmintic treatment; however parasite resistance to anthelmintic compounds is becoming an ever-increasing problem worldwide. Thus alternative control measures are now sought. This thesis comprises a number of studies which aim to investigate the genetic control of several aspects of the host response, and thus the application of such knowledge to develop alternative control strategies for gastrointestinal parasites in a commercial sheep population.

Analysis of data from 6-month old Scottish Blackface lambs exposed to a mixed, natural nematode infection demonstrated that the indicator traits, faecal egg counts (FEC), immunoglobulin A activity, eosinophil count, plasma pepsinogen activity and fructosamine concentration, investigated at 6 months of age were highly heritable and strongly correlated with the worm development traits. For example at a mean age of 22 weeks the heritabilities (± SE) for fructosamine concentration, IgA activity, eosinophil count and pepsinogen activity were 0.39 ± 0.16, 0.57 ± 0.15, 0.35 ± 0.15 and 0.56 ± 0.16 respectively. Strong negative genetic correlations (<-0.6) were often observed between worm development traits and eosinophil count, IgA activity and pepsinogen activity. A substantial genetic correlation was also observed between fructosamine concentration and worm length (0.67). However when such correlations were investigated across the 6-month time-period, the genetic correlations changed systematically and dramatically over time. For example, for all worm development traits, genetic correlations with eosinophil count were initially positive and moderate to strong, and then declined dramatically eventually becoming moderate to strong and negative at 5 months of age. These results provide an insight into the evolution of the genetic basis of the host parasite interaction at a time when the host immune response is developing, and help to define optimal measurement ages for selection purposes.

Two quantitative trait loci (QTL) studies were carried out on populations comprising different breeds and population structure; firstly a purebred Scottish Blackface flock and secondly a wide-breed cross flock developed from a

resistant breed, Gulf Coast Native, and a susceptible breed, Suffolk. Both studies identified QTL associated with parasitic resistance traits, and although there is no concordance between the results, this is possibly due to the animals being infected with different nematode species. In the Blackface study QTL associated with specific IgA activity were identified in chromosomes 3 and 20, in regions close to IFNG (chromosome 3) and the MHC (chromosome 20). QTL associated with Nematodirus FEC were identified on chromosomes 2, 3 and 14 and QTL associated with non-Nematodirus Strongyle FEC were identified on chromosomes 3 and 20. In the Suffolk x Gulf Coast Native study QTL associated with packed cell volume (PCV) were identified on chromosomes 1, 9 and 19 and with FEC on chromosomes 1, 6 and 19. QTL such as those identified in this thesis could be utilised in a marker assisted selection scheme to increase resistance to parasitic infection.

In the final study interactions between different parasite species within the host animal were investigated. Significant interactions were observed between *Cooperia* and *Teladorsagia circumcincta*, and *T. circumcincta* and *Trichostrongylus vitrinus*. Additionally *Cooperia* had a greater effect on FEC than *T. circumcincta.* The results from this study indicate that complex multi-parasitic relationships exist and hence when developing new control strategies it is essential to consider this background multi-parasitic infection and not simply focus on a specific species.

In conclusion this thesis provides evidence that many aspects of the host response are under some level of genetic control. Highly heritable indicator traits have been identified along with QTL associated with resistance traits, both of which could be utilised as selection criteria to increase the response to selection for resistance to gastrointestinal parasites within a commercial sheep population.

Chapter 1 Literature Review

1.1 Introduction

Intestinal parasites infect one quarter of the world's human population and are one of the most important causes of disease in domestic livestock (Chan 1997; Sykes 1994; McLeod 1995). Gastrointestinal parasites infect essentially all grazing livestock and are a major cause of economic loss to the agricultural industry. In sheep, gastrointestinal parasites cause a loss of production and subclinical infection may depress growth rate by as much as one third (Coop et al. 1985). The damage inflicted upon the host by the parasite is caused by a combination of events; the parasitized animal therefore exhibits decreased production, which is usually observed as a decreased growth rate and decreased wool weight.

It has been estimated that each year gastrointestinal parasites cost the UK sheep industry £85 million (Nieuwhof and Bishop, 2005). This cost can be broken down into two parts; lost income due to reduced growth (£63.7 million) and cost of treatment and control (£20.3 million), which can be further split into labour costs (£11.7 million) and cost of medicines (£8.6 million). When expressed as a cost per animal the estimated cost of gastrointestinal parasites is £4.70 per lamb. When compared to the cost of other major endemic diseases such as footrot (£0.15 per lamb) and sheep scab (£0.26) it becomes clear that gastrointestinal parasites are a major economic problem to the UK sheep industry.

Currently, parasites are predominantly controlled by the use of anthelmintics. Inevitably, however the use of anthelmintic compounds in livestock species has led to the evolution of anthelmintic-resistant parasites. Anthelmintic resistance is becoming an increasingly bigger problem for livestock

producers worldwide, as there have now been cases of resistance reported to every class of anthelmintic drug and at present no new classes are commercially available (Jackson and Coop, 2000; Waller, 1997). Anthelmintic treatment is expensive, as was demonstrated by the costs discussed previously (Nieuwhof and Bishop, 2005), and therefore repeated use increases the cost of livestock production. In addition, consumer concern regarding drug residues in food is also increasing and consequently demand is for drug-free produce. It is therefore imperative that new methods of parasite control are identified, firstly to overcome the problem of anthelmintic resistance, secondly to reduce the costs of livestock production and finally to meet consumer demands.

Alternative control measures include the use of sheep breeds with enhanced disease resistance (Preston and Allonby 1979; Courtney *et al.* 1985; Stear *et al.* 1990a; Gamble and Zajac 1992; Gruner *et al.* 1992; Stear and Murray 1994), selective breeding for increased resistance to infection (Albers *et al.* 1987; Gray 1987; Woolaston and Windon 2001), dietary protein supplementation (Coop and Holmes, 1996; Datta *et al.* 1999) and the use of nematophagous fungi (Waller *et al.* 1994). Vaccines are currently being developed but so far no vaccines against gastrointestinal nematodes are commercially available (Munn *et al.* 1993; Smith 1992; Smith 1999; Woolaston and Windon 2001).

The performance of domestic livestock populations has been improved through artificial selection on traits of economic importance. Selective breeding of domestic livestock for enhanced disease resistance is therefore becoming more interesting to breeders across the world. Resistance to gastrointestinal parasitism in sheep has been shown to be heritable (Albers *et al.* 1987; Bishop *et al* 1996; Bisset *et al* 1992; Douch *et al* 1995; McEwan *et al.* 1992 and 1995;

Morris *et al.* 1997a, b; Stear *et al.* 1997b; Woolaston and Piper 1996). Therefore selection for increased resistance, the ability of the host animal to suppress the establishment and/or subsequent development of a parasite infection, or resilience, the ability of the host animal to maintain relatively normal production while subjected to parasite challenge, to infection can be regarded as an alternative parasite control measure (Bisset and Morris 1996). In Australia, New Zealand and the UK breeders are striving to produce sheep with greater resistance to nematode infection (Bisset and Morris 1996; Woolaston and Baker 1996; Stear *et al.* 1997b; Bishop *et al* 2003). Breeding for enhanced disease resistance offers numerous advantages when compared with other methods of control. It can be an inexpensive and relatively simple way to improve animal health, welfare and productivity. As infectious organisms become resistant to the drugs used to control them (Nicholas 1987) and as the costs of treatment and veterinary care increase faster than the value of animals, then breeding for disease resistance becomes desirable. An increase in the number of resistant individuals will also benefit the entire flock as it will reduce pasture contamination due to there being fewer parasites within the resistant hosts and subsequently fewer eggs passing out with the faeces on to the pasture (Bishop and Stear, 2003). As pasture contamination decreases the number of larvae available for ingestion by the host animals will also decrease, hence parasite burdens and resulting production losses will be less problematic in animals with intermediate susceptibility levels.

1.2 Parasite Biology

There are many gastrointestinal parasitic nematodes that infect sheep, the most prevalent of which will be discussed briefly.

1.2.1 Nematodirus

Nematodirus are long, thin nematodes found in the host small intestine. Adults are 1 – 2cm in length with a thin anterior portion that enlarges at the front end of the worm, giving the head region a swollen appearance. There are four main species prevalent in sheep; these are *N. spathiger, N. battus, N. filicollis* and *N. abnormalis*. Distribution of *Nematodirus* is worldwide, however *N. battus* is the most pathogenic species found in the UK. Larvae are present on pasture within an eggshell and hence are very resistant to drying and freezing and may thus survive winter weather to infect sheep in early spring or late winter. Disease may be associated with developing larval stages of *N. battus* and may occur within 2 weeks of challenge. Heavy infection with *Nematodirus* is characterised by sudden onset of unthriftiness, profuse diarrhoea and marked dehydration (Merck Veterinary Manual, 2005). *N. battus* can cause death in lambs (Armour and Coop 1991; Noble et. al. 1989).

1.2.2 Cooperia

Cooperia are brownish-red nematodes, 4 – 6 mm in length with a swollen anterior end. They are found in the lining of the small intestine and their prevalence is worldwide. The exact consequence of this parasite in sheep is as yet not fully understood. The adult parasite is a blood sucker which penetrates the lining of the small intestine. Symptoms seen in lambs are diarrhoea, weight loss and decreased wool growth. Heavy infection may also cause a loss of appetite (Merck Veterinary Manual, 2005).

1.2.3 Trichostrongylus vitrinus

T. vitrinus are thin reddish-brown nematodes up to 5.5mm in length. Males have bursae with large lateral lobes. Adults are found in the host's small intestine. Infection with this parasite is seen in sheep worldwide. Host losses from *Trichostrongylus* infections can be significant and it is commonly seen in mixed infections. Acute infections in young animals cause weakness and sometimes death. Chronic infections cause wasting along with constipation or diarrhoea, the appetite may become depressed and anaemia may result as a combined effect of emaciation, diarrhoea and malnutrition (Merck Veterinary Manual, 2005).

1.2.4 Trichostrongylus axei

T. axei can be described as a stomach hair worm, the adult worm is very small, approximately 0.5 cm in length. They are found worldwide although the temperature and humidity levels must be sufficiently high for the third-stage larvae to be produced. The adult worms are found in the abomasum and occasionally the small intestine of the host. The adult worm may penetrate the lining of the abomasum causing irritation. Wart-like swellings may occur in these areas and cause diarrhoea and a reduced appetite. Heavy infection results in serious weight loss, hypoproteinemia and poor growth, particularly in mixed infections with *T. circumcincta* (Merck Veterinary Manual, 2005).

1.2.5 Haemonchus contortus

Haemonchus contortus is a relatively large nematode, which thrives in warm, humid conditions, as the L3 stage is vulnerable to freezing and drying out on pasture. This parasite is prevalent in subtropical regions, for example,

Australia, New Zealand and USA. Adults are 15 – 30 mm in length and are found in the host abomasum. They have a characteristic barber's pole appearance of entwined red stomach and white uterus. They are blood-sucking parasites that cause anaemia in the host, which can be fatal (Armour and Coop 1991; Noble *et al.* 1989). Haemonchosis in sheep is characterized by severe anaemia accompanied by generalised oedema. Hyperacute infection may cause death within 1 week of heavy infection. Chronic infection will exhibit the symptoms described and will be accompanied by progressive weight loss due to gastric dysfunction (Merck Veterinary Manual, 2005).

1.2.6 Teladorsagia circumcincta

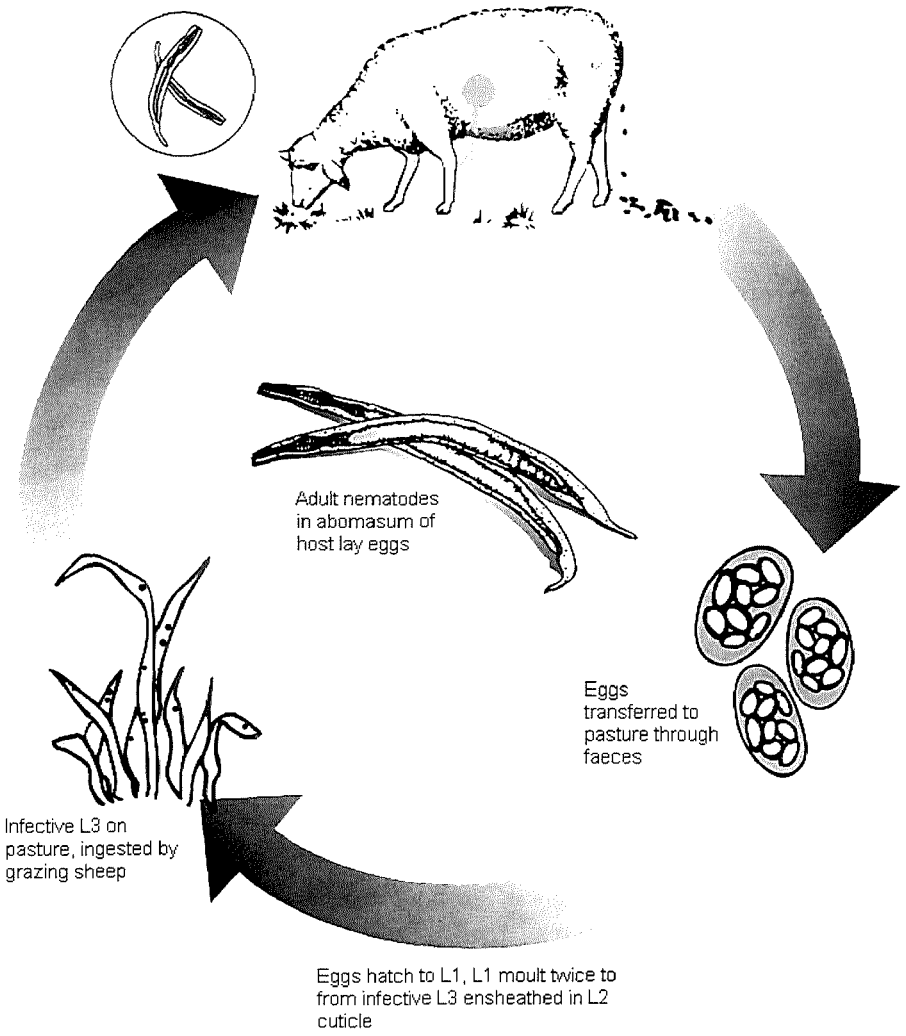
In this review the focus will be on the nematode *T. circumcincta. T. circumcincta* is the most prevalent and economically important nematode species within the flocks to be investigated, due to their location in Scotland. It has been reported that *T. circumcincta* accounts for three quarters of GI nematodes found in UK sheep flocks (Stear et al 1998).

T. circumcincta is a nematode parasite of small ruminants. It is a small, brown, thread-like worm that can be up to 12 mm in length. It is widespread among moist temperate regions of the world, particularly in cooler climates as L3 stage can survive freezing and relatively dry conditions on pasture. *T. circumcincta* is a prevalent and economically important parasite of sheep in Northern Europe (Armour and Coop 1991; Urquhart et al. 1991).

T. circumcincta has a typical direct nematode life cycle (Figure 1). Adult worms are located in the host abomasum; adult females lay eggs which pass out in the faeces where they hatch to give L1 larvae. Ten to fourteen days later the larvae moult twice to form the infective L3 larvae which are ensheathed in

the cuticle of the L2 stage. L3 larvae may survive on pasture for up to 4 months. Infection is by ingestion of these L3 larvae, once ingested they exsheath and within 1-3 days enter the gastric glands where a further moult is undergone to produce L4 larvae. L4 larvae often enter the wall of the abomasum where they become dormant (hypobiosis). Development to the L5 stage and subsequent maturation to adult may resume at any time after the minimal prepatent period of 17 days, and up to 3 months later (Urquhart et. al 1991). Figure 1.1 illustrates this life cycle (Whittier et al 1995).

Figure 1.1 Nematode Life Cycle



L3 larvae developing within the gastric glands distend the lumen of the glands and stretch the cellular lining, which results in mature functional parietal and peptic cells being superseded by undifferential, and therefore non-functioning, cells (Armour, Jarrett and Jennings 1966). Once embedded in the abomasal wall L4 larvae cause irritation and erosion of cells. Following such infection the parasite releases molecules that trigger mast cell degranulation. Mast cell proteases then break down the nearby cell junctions. A heavy or sustained infection leads to hypergastrinemia, which produces inappetance. Pepsinogen diffuses into the interstitial fluid and from there into the lymph and subsequently into the bloodstream. The breakdown of cell junctions allows protein in the interstitial fluid to flow into the gastrointestinal tract. The reduction in protein concentration in the interstitial fluid causes a loss of protein from the lymph and ultimately from the blood (Stear *et al.* 2003).

The relative protein deficiency observed as a result of infection has four causes: firstly infected animals eat less, secondly, the protein that they do eat is digested less efficiently. Thirdly the host proteins are lost into the gastrointestinal tract due to breaches in the epithelial barrier and finally infection causes an increase in protein demand as host protein is lost or diverted to immune and inflammatory responses (Stear *et al.* 2003).

As a result of the clinical signs described above *T. circumcincta* can have a major impact on the growth of young lambs. Infected lambs grow much more slowly than their uninfected contemporaries and only a proportion of this loss can be recovered by anthelmintic treatment (Coop *et al.* 1982; 1985). The major impact of nematode infection on lambs is a reduced growth rate during the first grazing season (Bishop et al. 1996). In growing lambs grazing improved

pasture, subclinical infection alone may depress growth rate by as much as one third (Coop et al. 1985).

1.2.7 Host – Parasite Interactions

Host-parasite interactions involving gastrointestinal parasites are overtly complex. Host resistance affects the biology of parasites in many respects, two key aspects being the reduction in reproductive output of the parasite and the reduction in parasite survival. When infected, the host will usually mount an immune response, however as parasites are organisms which have adapted to a parasitic existence, they have therefore evolved to minimise the impact of the host immune response. Yet, when observed, a group of animals will exhibit a range of susceptibility and much of this variation observed in the host response has been reported to be heritable (Bishop *et al.* 1996; Quinnell *et al* 2003; Stear *et al* 1997b). As the animals mature the acquired immune response develops. This response in the sheep is thought to vary between Th1-like responses in susceptible animals to a Th2-like response in resistant lambs. However, in mice it has been observed that the success of the immune response can be affected by the host's ability to mount a Th1 versus a Th2 response. One possible explanation is genetic variation among hosts in the production of cytokines that drive the host response.

In order to study host-parasite interactions in detail it is essential to harvest the parasites and larvae from the host animal. In the sheep this involves the animals being necropsied in order to recover the parasites from the gastrointestinal tract. Necropsy data generally consists of worm length, worm burden (adult and larval stages) and worm fecundity as indicated by the number of eggs *in utero*. Necropsy data is useful in developing an understanding of the

underlying genetic relationships, as it allows the parasite population measurements to be correlated to phenotypic trait measurements which can be observed before necropsy, thus creating a greater understanding of the parasite population within the host and how that is then reflected in the host phenotype.

Stear et al. (1999) reported a linear decline in worm length as worm numbers increase. For every extra thousand worms, the worm length declines by an average of 0.1mm. This could be explained simply as a result of crowding and competition for resources, or it may be that the increased number of worms stimulates an increased immune response. Although the number of worms has an important influence on worm length, it is not the only influence. There was little evidence in the study of Stear et al. (1997b) to suggest that variation among lambs in worm number is genetic in origin, the observed heritability was weak (0.14±0.10), and therefore it appears that most of the variation in worm number is non-genetic. However a much different situation became apparent for worm length. The heritability observed for worm length was extremely strong (0.62±0.20). This estimate implies that 62% of the observed variation was due to the average influence of host genes, almost twice as much of the variation in mean worm length as all other factors combined (Stear *et al.* 1997a;1999). It is a general belief of many parasitologists that longer nematodes are more fecund, both between and within species (Stear et al 1999). A strong phenotypic correlation has been observed between the number of eggs in the worm uterus and worm length (r=0.7), the heritability of the number of eggs *in utero* was estimated at 0.55±0.19, which is also very high (Stear *et al* 1997a). These heritabilities suggest that the major manifestation of genetic variation in resistance in 6-month old lambs is the control of worm growth and fecundity, not the control of worm numbers.

Stear *et al* (1999) propose two hypotheses; firstly, that immunity develops in two stages: initially, animals control worm growth and fecundity and then they control worm numbers, and secondly that sheep respond to all gastrointestinal nematodes producing an antibody response, followed by immediate hypersensitivity reactions. The effect of these responses will vary according to the nematode species involved in the infection.

Three major factors are associated with variation in worm length: worm number, IgA response to fourth stage larvae, and specificity of antibody response. When considered together these three factors account for a large proportion (>90%) of the variation in worm length (Stear *et al.* 1999).

1.3 Indicator Traits

In order to select animals for enhanced nematode resistance, we must first have the ability to identify susceptible and resistant animals. This identification may be possible by measuring a trait associated with the level of parasite infection, such as faecal egg count (FEC), immunological responsiveness, or ability to grow despite parasite challenge. Ideally such parameters would be easy to sample, be reliable and repeatable (Douch *et al.* 1996). In practical terms, breeders are currently limited to weighing sheep and wool harvested, taking faecal samples or taking blood samples. At present breeding values for such traits can be estimated using phenotypic measurements and pedigree information. However it may be possible to identify additional traits that could be useful as selection criteria, for example traits associated with immunological responsiveness.

Indicator traits are traits which provide an assessment of the infection or disease status of the host animal, for example FEC. FEC's are a measurement

of the number of eggs per gram of faeces being produced by the parasites within the host animal. It is thought that FEC provides a good indication of the parasite infection status of the host, as low FEC are thought to indicate low worm burdens and it is assumed that if there is a small number of eggs produced then there must be a small number of mature parasites within the host (Stear et al., 1995a). As an indicator trait FEC is used to rank animals which are given equal exposure to infection. The appropriateness of indicator traits is often assumed and more work is needed to confirm these assumptions. For nematode resistance it is necessary to confirm that the indicator traits provide a valid interpretation of the infection status of the host animal. The choice of indicator trait should make biological sense in terms of being representative of the disease in question (Bishop 2002). For resistance to nematodes it has been suggested that traits directly relating to immune response be measured as it is hypothesised that the immune response will impact on the infection status of the animal. Also of interest are traits which describe the impact of infection, these would be phenotypic changes caused by the infection e.g. pepsinogen levels.

The major drawback for all phenotypic indicators of resistance is that they require expression of resistance genes as a result of natural or artificial infection. Hence, for indicator traits to be utilised the animals must be infected and this infection status of the host will affect the animals performance. Performance of infected animals is not a measure of resistance, although it could be viewed as an indicator of tolerance or resilience. Tolerance can be defined as performance conditional upon infection status (Bishop 2002), as it is known that nematode infection of lambs decreases production due to the host redirecting its resources from growth into mounting an immune response. The necessity of artificially challenging sheep in areas where parasites are sporadic

adds to the cost and time taken for phenotypic assessment. However indicator traits may have the potential to directly indicate resistance, quantify immune response to infection and to quantify the consequences of infection.

1.3.1 Faecal egg counts

Faecal egg counts are the traditional disease indicator trait for investigation of gastrointestinal parasitic infections. Following natural, predominantly *T. circumcincta* infection, faecal egg counts are moderately heritable (Bishop *et al.* 1996; Bouix *et al.* 1998; Stear *et al* 1997b). Faecal egg counts indicate both adult nematode numbers and the mean fecundity of resident parasite populations. Per capita fecundity declines as the number of *T. circumcincta* in the abomasum increases (Stear and Bishop 1999). The relative importance of parasite number and parasite fecundity on egg production will depend upon the intensity of infection (Stear and Bishop 1999; Bishop and Stear 2000). In lambs the mean fecundity of *T. circumcincta* is much more heritable than differences in parasite numbers (Stear et al.1997b). Faecal egg counts are also relatively insensitive to changes in infection intensity (Bishop and Stear 2000).

A favourable genetic correlation has been observed between faecal egg counts and growth rate in many studies with values ranging from –0.1 to -0.80 (Albers *et al.* 1987; Bisset *et al.* 1992; Douch *et al* 1995; Eady 1998; Bishop and Stear 1999). This suggests that it would be straightforward to decrease faecal egg counts and increase growth rate simultaneously. This reduction in faecal egg count would then result in a reduction in pasture contamination. Reduced pasture contamination could result in a decreased parasitic challenge and thus increased performance for all animals subsequently grazing the same pasture

(Bishop and Stear, 2003). Therefore this indicates that a large part of the overall result is an epidemiological benefit of genetic improvement, as by increasing resistance of the flock exposure to infection is decreased. This combination of events suggests that selection for reduced FEC would lead to a much greater reduction in faecal egg counts than that predicted by classical quantitative genetic theory (Bishop and Stear, 1997).

Experimental breeding of sheep for nematode resistance has been based mostly on the selection of sheep for low FEC, because FEC is the easiest trait to measure and hence, the most widely studied trait at present. FEC can be influenced by factors such as the level of larval challenge, the species composition, worm burden and the degree to which worm establishment and adult fecundity is affected by the sheep's immune response. However, it is becoming apparent that selection based only on FEC may not be as reliable as has been suggested in the past. Several studies carried out under New Zealand conditions have reported negative genetic correlations between FEC and weight-gain parameters (Bisset *et al.*,1992; McEwan et al., 1992,1995). Douch *et al.* (1996) also suggest that under some circumstances low FEC comes at a cost in terms of production. Therefore the usefulness of FEC must be defined for the situation under investigation. Limitations to the usefulness of FEC include potential losses of production while anthelmintics are withheld, inability to store samples for long periods, and labour intensive nature of the measurement making automation unlikely.

1.3.2 Antibody Response

Many recent studies from the UK and New Zealand have suggested that a number of immunological parameters could serve as an alternative or

supplementary marker for resistance, as it is thought possible that FEC is markedly affected by the sheep's immune response (Douch et al., 1995; Stear et al., 2001a; Stear et al., 2002; Strain et al., 2002). Immunoglobulin A (IgA) represents the most prominent antibody class at mucosal surfaces, and is therefore of key importance in respiratory and gut infections (Macpherson et al 2000). In parasitized sheep in the UK, IgA is one of the most intensively investigated immunoglobulins. IgA activity is an indicator of antibody response to infection; it appears to be an important regulator of worm fecundity (Stear *et al.* 1995b). More specifically increased abomasal IgA activity against L4 has been associated with both increased larval inhibition and decreased adult size (Stear et al., 2004). The length of the adult female *T. circumcincta* is strongly associated with the number of eggs *in utero* and worm fecundity (Stear *et al.* 1995b; Stear and Bishop 1999; Strain *et al.* 2002). Deliberate challenge trials with *T. circumcincta* infections have indicated that an important immunological mechanism that regulates worm length and fecundity is the quantity and specificity of parasite-specific IgA (Stear *et al.* 1996a; McCririe *et al.* 1997;). Therefore the quantity and specificity of IgA may be a useful indicator trait for the identification of resistant and susceptible lambs.

Stear *et al.* (1996a) suggest that the ability of some sheep to regulate worm fecundity is due largely to strong IgA responses. These responses appear to be caused by a set of specific parasite molecules. It is suggested that all sheep do not recognize such molecules and hence variation in response is observed as the molecules do not cause a universal reaction. Seaton *et al.* (1989) demonstrated that sheep regulate worm length before they regulate worm burden when infected by *T. circumcincta*. Thus both studies suggest that sheep

may develop IgA responses which regulate parasitic infection, specifically worm fecundity.

Strain *et al.* (2002) estimated heritability of IgA response to be 0.56. This high heritability estimate is consistent with the theory that IgA regulates worm growth and fecundity and with previous findings of high estimated heritabilities for worm length and worm fecundity (Stear *et al.* 1997a; 1997b). Potential quantitative trait loci influencing IgA levels in humans have been located on chromosomes 10 and 13 (Wiltshire *et al.* 1998).

An association between IgA and worm number has been observed, but this has been found to be inconsistent. Hence IgA activity cannot be used to estimate worm numbers (Strain *et al.* 2002; Stear *et al.* 1995b). However Strain *et al.* (2002) did produce results, under conditions of natural challenge, that confirmed the previously observed associations between IgA and worm number. In the same study under deliberate challenge conditions, an association was observed between increased IgA activity and reduced mean length of adult female *T. circumcincta* and also between the number of adult nematodes and female adult worm length (Stear *et al.* 1995b).

In studies involving parasites other than *T. circumcincta,* IgG_1 and IgE have been more widely studied than IgA. In Romney sheep in New Zealand IgE specific to *T. colubriformis* has been reported to be negatively correlated with FEC and unfavourably correlated with production traits (Shaw et al., 1999). A negative phenotypic correlation has also been reported between serum IgE level and worm burden for *H. contortus* (Kooyman et al., 1997). Douch et al. (1995) investigated IgG_1 responses in the same Romney flock as previously described; this study reported very low genetic correlations between IgG_1 and production traits. Thus it appears that the antibody studied changes with the parasitic

species under investigation. The reasons for this are unclear but may be due to the type of response triggered by the parasite or historical reasons relating to previous research.

Therefore it appears that it may be possible to selectively breed for elevated antibody levels. Under conditions of low nematode challenge it may possible that antibody responses could provide a better indicator of resistance than FEC (Douch *et al* 1996). Since IgA is heritable and associated with resistance to deliberate infection (Smith *et al.* 1985; Stear *et al.* 1995b) as well as natural infection (Strain *et al.*2002), it appears to be a possible indicator trait for resistance to infection with *T. circumcincta*.

1.3.3 Fructosamines

Fructosamine concentrations reflect the protein status of the animal. Infection with *T. circumcincta* can induce a relative protein deficiency and it has been suggested that fructosamines could be utilised as indicators of the severity of infection. Fructosamines are stable covalent ketoamines formed by the nonenzymatic reaction of glucose with amino groups on proteins (Bernstein 1987). Protein turnover increases with nematode infection and Heath and Connan (1991) observed a decrease in fructosamine concentrations following deliberate gastrointestinal infection.

Stear *et al.* (2001a) reported that variation in fructosamine concentrations was moderately heritable (0.34 ± 0.14). When measurements were taken at the same time point decreased fructosamine concentrations were associated with decreased bodyweight, decreased faecal egg counts in some years, increased pepsinogen responses and increased parasite-specific IgA responses. Lambs with low fructosamine concentrations subsequently acquired more nematodes of

all species and had shorter adult female *T. circumcincta*. Hence fructosamine concentrations were associated with current levels of infection and also appeared to predict future levels of infection. Associations between fructosamines and faecal egg output were not consistent in this study. The ability to control the number of *T. circumcincta* that establish appears to be largely or wholly acquired by sheep after the first grazing season (Stear *et al.* 1999a). Therefore the association between fructosamine concentrations and worm numbers is unlikely to be solely due to variation in immune responsiveness although a role for the immune responses cannot be ignored.

Grazing behaviour could also be responsible for the observation that lambs with low fructosamine concentrations acquire more nematodes after anthelmintic treatment. It has been suggested that lambs with low fructosamine concentrations might graze lush areas in an attempt to restore their relative protein status; as faeces can act as a fertilizer, lush grass may be more heavily contaminated with nematodes (Hutchings *et al.*1998, 1999).

Because fructosamine concentrations are repeatable, moderately heritable and associated with a variety of parasitological variables, they appear to be useful indicators for estimating the severity of infection from natural, predominantly *T. circumcincta* infection. However the association between the severity of infection and fructosamine concentrations appears to be relatively complex. Therefore further work is necessary to understand this association and also the relationships between fructosamines and other indicator traits. Such understanding would enable the full potential of fructosamines to be utilised as an indicator of parasite infection.

1.3.4 Pepsinogens

Pepsinogen is the precursor of the digestive enzyme pepsin. Pepsinogen is converted to pepsin only in acidic conditions. During parasitic challenge the pH of the gut can increase from pH2 to pH7, thus neutralising the gut and preventing pepsin from being formed, thereby increasing the concentration of pepsinogen. An increase in pepsinogen therefore may indicate the presence of parasites and resultant damage to the gut of the host animal (McKellar *et al.* 1986; Fox *et al* 1989). Stear *et al* (1995c) stated that plasma pepsinogen concentrations are reasonably reliable, as they show high repeatabilities between samples; these were 0.87 in the period 5-8 weeks after infection and 0.64 in the period 1-4 weeks after infection (samples taken at weekly intervals). This study concluded that under experimental conditions FEC's, peripheral eosinophil counts (described below) and plasma pepsinogen concentrations provide a reasonable estimate of the worm burden and that use of the three traits concurrently could enable more effective identification of resistant and susceptible animals.

1.3.5 Eosinophils

Eosinophils are a type of white blood cell. Traditionally assumed to have a major role in the innate immune system response, the function of eosinophils in the response to parasitic infection is as yet not clearly defined. The mobilisation of eosinophils to the site of infection is linked to the Th2 response; however the ability of the eosinophil to destroy parasites is largely due to it having an Fc receptor, this being the receptor which allows the eosinophil to bind to IgA molecules on the surface of the parasite. The role of eosinophils in the immune response to nematode infection has not yet been determined; they can function as effector cells (Walsh 1999; Meeusen and Balic 2000; Behm and

Ovington 2000) and also could possibly be a by-product of the processes required to control nematode infections.

Eosinophilia is the condition of having an increase in the number of eosinophils in the circulating blood. Within flocks of sheep deliberately infected with *T. circumcincta* local and peripheral eosinophilia (Stear *et al.* 1995c; Doligalska, Moskwa and Stear 1999) along with eosinophil-related responses (Stevenson *et al.* 1994) are associated with resistance to infection. It has also been observed that eosinophilia is often, but not always, higher in sheep that are resistant to the nematodes *Trichostrongylus colubriformis* (Kimambo, Macrae and Dewey 1988; Dawkins, Windon and Eagleson 1989; Rothwell *et al.* 1993) and *Haemonchus contortus* (Gill 1991; Woolaston *et al.* 1996), as well as sheep selected for resistance to natural infection in New Zealand (Douch *et al.* 1996).

Stear *et al.* (2002) reported a study to investigate the value for peripheral eosinophilia as an indicator of resistance to *T. circumcincta* and to explore possible reasons for the apparent inconsistency of the associations between eosinophilia and faecal egg counts. They found that eosinophil counts were moderately to strongly heritable, in August heritability was 0.48±0.16 and in September it was 0.43±0.17, with results appearing similar to and possibly slightly higher than the heritability of faecal egg counts. The heritability estimates were somewhat higher than 0.19 ± 0.08 in Merino sheep deliberately infected with *Trichostrongylus colubriformis* (Woolaston *et al.* 1996). Stear *et al.* (2002) also stated that eosinophil counts were consistently related to various parasitological measurements. Although eosinophil counts are unlikely to replace faecal egg counts as a marker of resistance, they may supplement egg counts, especially in predominantly *T. circumcincta* infections where the information provided by faecal egg counts is useful but limited (Bishop and Stear

2000). Associations between faecal egg counts and eosinophil concentrations were only found to be present in older lambs (Stear *et al.* 2002), however Douch *et al.* (1996) suggested that any association between eosinophil counts and faecal egg counts may only become apparent when eosinophil counts are elevated. As eosinophil levels can fall quite rapidly post anthelmintic treatment (Stear *et al.* 1995c) caution must be taken if eosinophils are to be used as an indicator trait for resistance. Hence eosinophil concentrations may be a useful indicator of resistance to nematode infection but only in older lambs that have been continually exposed to infection.

1.4 Detection and utility of QTL

In the absence of new anthelmintic compounds or commercially available vaccines, one of the first responses in finding a solution to the anthelmintic resistance problem has been the development of selective breeding schemes for parasite resistance in the UK, Australia and New Zealand. However these schemes are based on selection for resistance using indicator traits, for example, faecal egg counts (FEC), as previously discussed. However, collecting and quantifying an indicator trait such as faecal egg count is a costly and time consuming process and also requires the animal to undergo parasitic challenge.

A Quantitative trait locus (QTL) is a region of a chromosome that is associated with a particular trait (e.g., liveweight). Though not necessarily genes themselves, QTLs are stretches of DNA that are closely linked to the genes that underlie the trait in question. The discovery of QTL for disease resistance traits, for example FEC and IgA, would enable these traits to be selected upon within a breeding scheme with the aim of increasing resistance at the flock level. This

method of selection may enable selection in the absence of infection, thus not adversely affecting production traits as would be the case with indicator traits as then infection is necessary for the traits to be measured. Selection based on QTL may also enable potentially faster responses to selection. The identification of genes which regulate resistance would improve understanding of the mechanisms of host defence against parasites and could also accelerate the development of efficient vaccines.

QTL for nematode resistance have only been attempted using faecal egg counts, as the hypothesis is that reduced egg counts are an appropriate measure of resistance. However when used as an indicator trait the informativeness of FEC can vary between years, therefore QTL could be used for selection and in theory 'bridge the gap' in the selection data. Moreover utilisation of QTL would enable selection of sheep at any age and irrespective of their history of parasite exposure. Therefore, it would be a very useful method as it may be possible to select directly for parasite resistance without prior infection using QTL within a selective breeding scheme.

There are two commonly used methods of QTL detection which will be discussed; the linkage mapping method, using whole or partial genome scans, and the candidate region method.

1.4.1 Linkage Mapping Method

The first method to be discussed here is the linkage mapping method, which may involve whole or partial genome scans. The main benefit of this method is the inclusion of large chromosomal regions, and potentially the whole genome, however this is sometimes achieved with very sparse marker coverage. To attain dense marker coverage across the entire genome would

prove very costly. Several studies have utilised this method and their findings will be summarised.

Beh *et al.* (2002) performed a genome wide scan to dissect the genetic basis of gastrointestinal parasite resistance in sheep. This study presents no convincing evidence for the presence of major genes affecting *T. colubriformis* FEC in the population of sheep studied, but does present putative QTL positions associated with FEC traits on chromosomes 1, 2 and 6.

Shay *et al* (2002) performed a partial genome scan to identify chromosomal regions in the ovine genome that are associated with resistance to gastrointestinal parasites, in an F2 sheep population segregating for parasite burden, produced from a cross between Gulf Coast native (resistant) and Suffolk (susceptible) breeds. Preliminary QTLs for parasite resistance were identified on chromosomes 1, 3 and 19. Chromosome 19 yielded the most significant results with a putative QTL localized at the proximal end of the chromosome.

Moreno et al. (2006) performed a genome scan on two populations; Firstly a backcross Sarda x Lacaune population and secondly a backcross BlackBelly x INRA 401 population, both of which were exposed to a natural mixed infection. Overall 34 QTL were detected at a genome-wide threshold relating to FEC and PCV traits. Several QTL on chromosomes 3, 12 and 13 were detected in the same regions for more than two traits in both populations. This study also reported that different QTL were observed when different challenge methods were used.

Finally in this section a QTL study was carried out under Spanish commercial conditions on the Churra population (GENESHEEPSAFETY 2006) This study aimed to identify QTL segregating within commercial breeding programmes, in adult lactating animals. A full genome scan was performed

which returned QTL at a genome–wide threshold on chromosomes 1,6, 9, 10, 13, 14, 20 and 26, associated with serum pepsinogen levels, IgA and FEC. This study is very informative as it provides a wealth of knowledge on QTL's associated with resistance to parasitic infection segregating in the adult lactating ewe.

From the descriptions above it appears that many QTL have been identified for traits associated with parasitic resistance however these involve a wide-variety of breeds and parasitic species, thus at present there is little common ground for comparison.

1.4.2 Candidate Gene Method

This method is based on QTL methods that focus on candidate genes; these are genes thought to be associated with the trait in question based on information from previous studies. This method covers relatively small sections of chromosomes surrounding candidate genes, with a high marker density. Two candidate gene regions, which are thought to be associated with parasitic infection in sheep, are the MHC and IFNG (Schwaiger *et al.*1995: Coltman *et al.* 2001).

1.4.2.1 Major Histocompatibility Complex (MHC)

One candidate region for genes involved in parasite resistance or susceptibility is the MHC, because genes of this complex encode molecules that play a central role in antigen presentation to T-lymphocytes and thus the immune response to parasitic infection (Zinkernagel and Doherty 1979). The MHC plays a major role in determining the specificity of the antibody response to nematodes (Kennedy 1989). The extensive polymorphism at class I and class II

loci is associated with variation in immune responsiveness to a variety of organisms, including parasites (Wakelin and Blackwell 1988). Associations between the MHC and nematode infection have been reported in several species, cattle (Stear *et al.* 1988;1990b), guinea pigs (Geczy and Rothwell 1981), mice (Else *et al.* 1990), pigs (Lunney and Murrell 1988) and sheep (Outteridge *et al.* 1985; 1986; 1988; Luffau *et al.* 1990; Hulme *et al.* 1993; GENESHEEPSAFETY 2006).

Schwaiger *et al.* (1995) reported an association between the MHC and faecal egg counts at some time points in a population of Scottish Blackface sheep. This association was linked to the DRB1 locus within the MHC region. It has been suggested that DR molecules are directly involved in regulating resistance against gastrointestinal nematodes because of their central role in antigen presentation and antibody responsiveness. However the MHC includes a large number of polymorphic loci in linkage disequilibrium and it is possible that allelic variation at a different gene closely linked to the Ovar-DRB1 locus could be responsible for observed association. This study was followed up by Stear *et al* (1996) investigating the association between MHC class I molecules and faecal egg counts in a separate set of lambs on the same farm. Significant differences were observed in lymphocyte antigen frequencies. Tight linkage and strong associations were reported between these lymphocyte antigens and polymorphisms within the ovine class II regions. There were also significant associations between lymphocyte antigens and DRB1 polymorphisms, and the presence of antigen G13br and reduced faecal egg counts. As lymphocyte antigen G13br is in linkage disequilibrium with DRB1 allele g2 which has also been associated with reduced faecal egg counts, this result provides partial confirmation of the relationship between polymorphism within the ovine MHC

and reduced faecal egg counts. It is possible that the putative class I antigens are less strongly associated than the DRB1 alleles because DRB1 is more closely linked to, or perhaps may be, the disease-susceptibility locus (Stear *et al.* 1996a). Three associations were also reported, within the MHC regions in the study of a Roehnschaf flock, associated with haematocrit level (CP73), IgL level (DYMS1) and FEC (BM1815) following an artificial challenge with *Haemonchus contortus* (Janssen et al 2002).

1.4.2.2 Interferon Gamma

Several studies have identified QTL associated with parasite resistance on chromosome 3 (Beh et al 2002; Coltman et al 2001; Paterson et al 2001). These studies have utilised both candidate gene and linkage methods, however for the purpose of this review they will all be discussed below.

Crawford and McEwan (1998) suggested that the IFNG locus is associated with variation in parasite resistance in domestic sheep. Coltman *et al* (2001) investigated if microsatellite polymorphism at the o(IFN)-γ locus (Schmidt *et al.* 1996) was associated with parasite resistance in a free-living population of naturally-parasitized Soay sheep on the island of Hirta, St Kilda. In this study faecal egg counts were found to be significantly associated with alleles at the o(IFN)-γ locus, suggesting that there may be a QTL associated with reduced FEC segregating near the (IFN)-γ gene in Soay sheep. A QTL in the interval IFNG – BMS1617 was suggested for a multispecies challenge in Romney divergent selection lines (Paterson et al 2001), and a QTL associated with T. colubriformis challenge was identified in Merino selection lines (Beh et al 2002). Together these results suggest that there may be a gene close to IFNG that influences resistance to several species of nematode.

1.5 QTL studies in mice

QTL associated with gastrointestinal nematode burdens have also been identified in mice. Iraqi et al (2003) conducted a study based on an F2 population created from a susceptible x resistant strain cross. This study initially used a genome-wide scan using 4-5 microsatellite markers per chromosome to genotype the most resistant and the most susceptible animals. Once this initial genotyping was analysed more animals were genotyped at regions of interest. Significant QTL were reported on chromosomes 1, 2, 8, 13, 17 and 19, associated with either FEC or worm burden. At present it is not possible to draw direct inferences between the mouse and sheep QTL studies. However the mouse provides an interesting model which ultimately, as comparative genomics progresses, may provide information to further knowledge on gastrointestinal parasite resistance in sheep.

1.6 Microarray Studies

Microarray analysis is a relatively new technology which simultaneously analyses gene expression from potentially thousands of genes. At present there is only one published study which uses microarray technology to investigate gene expression associated with nematode parasite resistance (Diez-Tascon et al 2004). This study compares susceptible and resistant animals from lines selected for high and low faecal egg count since 1986. The experiment examined gene expression in duodenum tissue from four resistant and four susceptible lambs, all of which had been exposed to a natural mixed infection, and identified over 100 genes that were differentially expressed. Of the genes identified, seven that were upregulated in the resistant animals and six that were upregulated in the susceptible animals fall under an already characterised QTL.

In this study two main functional themes emerged during the gene identification process. These were antigen processing and presentation and muscle function. These themes suggest that the function of the genes identified as differentially expressed could be linked to immune response and increased gut muscle function. In particular this may be associated with enhanced contractility of intestinal muscle which may aid expulsion of parasites. This study provides an interesting insight into how microarray technology can be utilised within disease resistance studies in order to identify potential genes of interest for further study; particularly those which may be influencing host resistance mechanisms.

1.7 Marker Assisted Selection

Following successful identification of a QTL for parasite resistance a marker assisted selection (MAS) scheme could be implemented to improve flock resistance. Marker-assisted selection plays a prominent role in the field of plant breeding, however examples of successful, practical outcomes in livestock species are rare. It is clear that genetic markers hold great promise, but realising their potential remains elusive in animal breeding. The main advantage with MAS for parasite resistance is the ability to select animals prior to infection.

In order to implement a MAS scheme there are 4 basic steps to follow:

1) Search for genetic markers
2) Establishment of a linkage map of the markers
3) Detection of association between QTL and markers
4) Use of markers in a breeding program

(Meuwissen and Van Arendonk, 1992)

From the literature previously discussed it would appear that at present work on QTL for parasite resistance is at step 3. More research is necessary in order to

provide a greater understanding of host-parasite interactions and thus more accurate QTL. However given the depth of work already published and that which is known to be underway a MAS scheme to include parasite resistance traits is a possibility for the near future.

As there is little published evidence available on MAS in livestock disease it is only possible here to discuss models. Van der Waaij *et al.* (2002) investigated a model which evaluated the use of QTL for disease resistance in selection for increased production compared to mass selection on observed production, under both constant and intermittent infection pressure. In an earlier study van der Waaij *et al* (2000) concluded that it is not necessary to measure the level of infection in order to increase it because if animals are selected on the basis of observed production in an infected environment then the selection pressure is indirectly for resistance. The disadvantage of the earlier model was that exposure to the pathogen would be required in order to obtain phenotypic observations for observed production. The model developed in van der Waaij *et al.* (2002) used selection on predicted production by making use of genetic markers that were linked to multiple QTLs affecting resistance. The results of the study indicate that MAS could be a good alternative to mass selection (observed production) for increasing production and resistance simultaneously (Van der Waaij *et al* 2002).

The models described indicate the potential that MAS holds for livestock improvement programmes. However an environment in which the infection (parasite) under consideration is absent may also differ in some other constant environmental factors compared to the infected environment, therefore it is important that QTL are mapped in the environment in which the selected animals are to be kept (van der Waaij *et al.* 2002).

Prospects for MAS are greater for traits that are difficult to improve through conventional methods. Selection must be based on a combination of marker and phenotypic data. However until complex traits, such as parasite resistance, can be fully dissected, the application of MAS will be limited to genes of moderate to large effect and applications that do not endanger the response to conventional selection. Therefore although MAS holds great promise for genetic improvement, until further research is carried out the observable phenotype will remain an important component of genetic improvement programmes, due to its ability to account for the collective effect of all genes (Dekkers 2002).

1.8 Conclusion

In conclusion it appears that worldwide the sheep industry is in vital need of alternative strategies for the control of nematode infection. Alternative strategies must have the potential to reduce the frequency of anthelmintic treatment, hence slowing further development of anthelmintic resistance. This would result in a decrease of the production costs associated with parasitic challenge and also satisfy the growing consumer concern with drug residues in produce.

It is thought that indicator traits may have the potential to provide a cost effective and reliable selection process. This would enable breeders to implement an economic selective breeding programme for parasite resistance. However in order to utilise indicator traits the animals must first be subjected to parasitic infection. Further research is required into how indicator traits reflect the parasite population within the host and also how they are best combined to form a selection index. In order to create a selection index it is essential to first

have key information such as heritabilities, genetic correlations and longitudinal changes in these values, if these changes are significant then it becomes necessary to determine the optimum age for selection.

In order to select animals without them first undergoing parasitic infection, QTL detection and subsequent implementation of MAS may provide an ideal solution. However QTL detection is costly as is the successful development and subsequent monitoring of a MAS programme. In addition effects on production traits must be determined before selection for disease resistance can begin. This is definitely an area for more research as promising results have been published (Beh et al 2002; Diez-Tascon et al 2002; Janssen et al 2002; Coltman et al 2001; Schwaiger et al 1995). These studies suggest that there may be genes at or close to MHC and IFNG which influence nematode resistance, but there is little consensus in these results due to the use of diverse breeds and differing parasite species, hence reliable results are not available at present to enable this to be utilised in a commercial flock. Hence, larger datasets are required to further study this potential area for improving nematode resistance.

1.9 Objectives

This thesis aims to investigate factors that will determine the feasibility of breeding sheep for enhanced nematode resistance. From the literature it is apparent that further investigation is required into alternative strategies to manage the gastrointestinal parasite problem in commercial sheep. In order to investigate nematode control strategies it is essential to gain a greater understanding of the genetic control of resistance to nematode infection and to develop selective breeding schemes. This thesis had the following objectives:

1) To assess the potential ability of indicator traits to aid selection for resistance to nematode infection. This will involve describing the development of the genetic control of each trait with age, and to explore the genetic, phenotypic and environmental relationships between the parasitic traits and indicator traits.

2) To investigate longitudinal changes in the genetic control of immunological parameters associated with parasitic infection, and their relationship with observed parasite burdens. This analysis will indicate if there is an optimum age for selection of animals based on these traits.

3) To identify QTL associated with nematode parasite resistance that are segregating in a Scottish Blackface flock which is not under selection for resistance traits. QTL would enable selection of animals for increased parasite resistance without previous infection.

4) To identify QTL associated with resistance to parasitic infection, that are segregating in the F_2 progeny of a resistant (Gulf Coast Native) x susceptible (Suffolk) crossbreed developed from a backcross design. This study population provides a good comparison to the flock investigated in chapter 4. It may also provide independent verification of results as it enables a comparison within this thesis of an flock unselected for parasite resistance with an experimental cross involving naturally resistant and susceptible breeds.

5) To investigate the relationships among different parasite species within the host animal. This analysis aims to investigate if these relationships are present

and if so the size of the effect and the direction if between parasites in different locations. In addition to the effect of parasite burden on these interactions the effects of parasite presence or absence will also be investigated. Finally the effect of the different parasites species on faecal egg count will be analysed.

Each objective described above will comprise a separate chapter within this thesis. The combination of results from these investigations will enable us to provide many answers to the problem of gastrointestinal parasites. These results will provide a greater understanding of host – parasite interactions, with particular reference to genetic control, along with parasite species interactions within the host animal. This knowledge will then provide a better understanding of the underlying biology of parasitic infection, the resulting host immune response and ultimately the traits we wish to utilise in selective breeding schemes either by using an index or through MAS using QTL.

Chapter 2 Genetic relationships between indicator traits and nematode parasite infection levels in 6 month old lambs

2.1 Introduction

The first objective of this thesis is to assess the potential ability of indicator traits to aid selection of animals for resistance to nematode infection. If selective breeding for nematode resistance is to be implemented as an alternative nematode control strategy then it is necessary to be able to quantify resistance. Faecal egg count (FEC), the number of eggs per gram of faeces, is the indicator trait commonly used to assess the level of infection. Faecal egg counts indicate the product of the adult nematode numbers and the mean fecundity of resident parasite population. However, for *T. circumcincta* infections faecal egg counts appear to be relatively insensitive to changes in infection intensity (Bishop and Stear 2000), and additional indicator traits may aid the identification of resistant and susceptible sheep.

There are various indicator traits that may be used to assess disease resistance, as described in Chapter 1. In addition to faecal egg count these indicator traits include immunoglobulin A (IgA) activity, eosinophil counts, pepsinogen activity and fructosamine concentration. Immunoglobulin A (IgA) is a secreted antibody which is part of the acquired immune response. It has a major role in gut infections and appears to regulate worm fecundity (Stear *et al.* 1995b; Smith *et al.* 1985). Pepsinogen is a precursor of the digestive enzyme pepsin. An increase in pepsinogen activity is indicative of a rise in the pH of the abomasum. *T. circumcincta* can cause the pH of the abomasum to rise, preventing the formation of pepsin. An increase in pepsinogen activity therefore indicates the presence of parasites and resultant damage to the gut of the host animal (McKellar *et al.* 1986; Fox *et al.* 1989). Fructosamine concentration reflects average glucose and protein concentrations as well as the rate of protein turnover. *T. circumcincta* can cause a relative protein deficiency as well

as an increase in protein turnover. Heath and Connan (1991) observed a decrease in fructosamine concentration following deliberate gastrointestinal infection, while Stear *et al.* (2001) reported that naturally infected animals with low fructosamine concentrations subsequently acquired more nematodes of all species and had shorter, less fecund, female *T. circumcincta.* Eosinophils are a type of white blood cell and are part of the immune response. Changes in eosinophil counts have been associated with resistance to parasitic infection, and they may interact with IgA to regulate nematode growth (Doligalska, Moskwa and Stear 1999; Stevenson *et al.* 1994; Stear *et al.* 2002).

Therefore, the objective of this chapter is to describe the development of the genetic control of each of these traits with age, and to explore the genetic, phenotypic and environmental relationships between the parasite infection and indicator traits in 20 – 24 week old lambs expressing large genetic differences in resistance.

2.2 Materials and Method

2.2.1 Animals

Approximately 1000 Scottish Blackface lambs, predominantly twins, were studied over a five-year period (1992-1996). The animals were bred from 38 sires and 505 dams, some of which were used across years; Table 2.1 shows the number of parents for each year. Single-sire mating was used such that sire and dam could be assigned to all progeny. The animals were kept on a commercial farm in southwest Strathclyde, Scotland. Husbandry procedures

followed standard commercial practice. All lambs were born outside during the final two weeks of April and the first week of May and were continuously exposed to natural mixed nematode infections whilst grazing. Anthelmintic treatment (albendazole sulphoxide) was administered, at the dosage rate recommended by the manufacturer, every 28 days from 4 to 20 weeks of age. Blood samples were collected every 28 days from 4 to 24 weeks of age. Blood samples were not collected in October 1992, October 1993 and August 1995. Approximately half of each cohort was necropsied at 6-7 months of age (6 weeks after final anthelmintic treatment) in 1992-1995. The number of measurements collected for each trait are summarised in Tables 2.2 and 2.3.

2.2.2 Faecal Egg Count

Faecal samples were collected from the rectum of the lamb at 4 weeks of age and every 28 days until 24 weeks. Faecal egg counts were made from a 3g sample of faeces using the modified McMaster technique (Gordon and Whitlock, 1939; Bairden 1991). Each egg counted represented 50 eggs per gram of faeces. In 1993 duplicate aliquots were counted and in 1994 and 1995 quadruplicate samples were counted in these years each egg counted represented 25 or 12.5 eggs per gram of faeces, respectively. A full description of the analysis of a subset of this dataset has been provided by Bishop *et al.* (1996).

2.2.3 Fructosamine Concentrations

The fructosamine concentration in plasma samples was measured using a Cobas Mira discrete biochemical analyser with a commercial kit (Unimate fructosamine) and calibrated with a specific glycated polylysine calibrant, all of which were obtained from the same supplier (Roche Diagnostics Ltd.). Fructosamine concentrations (µmol/l) were measured in July, August and September 1992, September 1993 and September 1994. Further details have been provided by Stear *et al.* (2001a).

2.2.4 Eosinophil Counts

To estimate the concentration of eosinophils in peripheral blood, 10 µl of whole blood was added to 90 µl of Carpentier's solution (Dawkins *et al.* 1989), left for 5 minutes and duplicate samples counted on a haemocytometer. Each cell counted represented 5.6 cells / µl of whole blood. Eosinophil concentrations were measured in May, June, July, August and September 1993 and August and September 1994. Further details have been provided by Stear *et al.* (2002).

2.2.5 Immunoglobulin A levels

The activity of plasma Immunoglobulin A (IgA) against a somatic extract of 4th-stage larvae from *T. circumcincta* was measured by indirect ELISA, as described by Strain *et al.* (2002). Relative IgA activity was measured as: (observed – standard) / (high control – standard), where the observed value is the sample mean from 3 replicates for the animal, the standard is the mean of 3 replicates from a pooled sample of helminth-naive lambs and the high control is the mean of 3 replicates from a pool of high-responder lambs (Sinski *et al.*

1995). The pool of high responder lambs was created by combining equal quantities of plasma from 6 lambs that gave strong IgA responses following natural infection. The value for each animal was therefore expressed as a proportion of a positive control. IgA levels were measured in August and September and October of each year with the exception of October 1992 and 1993 and August 1995.

2.2.6 Pepsinogen Concentrations

Plasma pepsinogen concentrations were measured using the method of Paynter (1992), which was adapted for small quantities, as described by Stear *et al.* (1999). To simplify comparisons I have presented the pepsinogen concentrations as a percentage of the internal standard. Pepsinogen concentrations were measured in September of each year except 1996.

2.2.7 Parasitological assessments

At slaughter (6-7 months of age) the abomasum was removed, opened along the greater curvature and washed with tap water under moderate pressure. The contents and washings were made up to 2 l, from which ten 4 ml aliquots were examined to estimate the size of the adult nematode population. The mucosa from one half of the washed abomasum was digested with pepsin-HCl for 6 hours at 42 °C; the digest was then made up to 2 l and a 0.02 sub sample was used to estimate the number of larvae (Armour, Jarrett and Jennings, 1966). Worm length was observed by collecting and measuring a sample of at least 25 female worms from each animal, 'eggs *in utero'* was estimated by counting the

number of eggs *in utero* for the same sample of females. Parasite data was collected in all years except 1996. The parasite traits recorded were worm length, number of eggs *in utero*, adult worm burden (number of males and females were also noted), number of L5 larvae and number of L4 larvae for all nematodes. However only the results for *T. circumcincta* have been presented here. These traits will be referred to as necropsy traits.

2.2.8 Data Analysis

Data analysis began with an examination of the distribution of the traits; all traits except worm length and fructosamine were skewed and were log transformed prior to further analysis. The transformations performed were: a straightforward log transformation for number of eggs *in utero*; *ln* (trait + x) where x = half of the measurement increment for worm burden and faecal egg count; and *ln* (trait + 1) for eosinophil and IgA. A restricted maximum likelihood package, ASREML (Gilmour et al., 1996) was used to perform univariate analysis with an animal model. The pedigree contained 1613 animals, including 38 sires and 505 dams. In the animal model the fixed effects fitted were year of birth, field grazed before weaning, sex, day of birth (fitted as a continuous effect) and type of birth (twin or single), along with significant two-way interactions. Measurements of the same variable taken at different time points were analysed as separate traits. Heritability estimates were then calculated for each trait from the univariate analysis. This analysis was repeated fitting a litter effect (c^2), litter classes were coded by dam/year and this was fitted as a random effect; the significance of the litter effect was tested using a likelihood ratio test. Bivariate analyses were also performed using ASREML to enable calculation of

environmental, phenotypic and genetic correlations between traits at ca. 6 months of age; an animal model was fitted with the same fixed effects as above. For traits where the univariate analysis had shown a significant litter effect, this effect was also fitted to the same trait in the bivariate analysis.

2.3. Results

Tables 2.2 and 2.3 contain summary data for all the traits investigated. Table 2.2 illustrates the range of infection levels observed within the flock and the range in worm development traits (worm length and eggs *in utero*). Worm length varied from 0.57cm to 1.22cm and the mean number of eggs *in utero* per lamb ranged from 1 to 67. Table 2.3 contains the summary data for the indicator traits; this also shows the number of measurements taken for each trait at each time point. The number of measurements varied considerably between traits, with relatively few measurements for eosinophilia and fructosamine concentration in very young animals.

Heritabilities for necropsy traits are shown in Table 2.4. These heritabilities have been previously estimated and published (Stear *et al.* 1997a) and are included here for completeness. The worm number traits appear to have low heritabilities, however the worm development traits, worm length and no. eggs *in utero*, are highly heritable in this dataset. Heritabilities for indicator traits are shown in Table 2.5. In general, the indicator traits were moderately to highly heritable. Differences were observed in heritabilities for the same trait across time points, however once the standard errors are taken into consideration, consistent time trends were not apparent. Significant litter effects were observed for pepsinogen and fructosamine concentrations. For fructosamine

concentrations the litter effect is large in early measurements and this coincides with lower heritability values.

The correlations between the necropsy traits are shown in Table 2.6. If the standard error of the genetic correlation exceeded 0.5, correlations were not presented. Genetic correlations were usually stronger than corresponding phenotypic correlations. In general terms, worm number traits were positively correlated with each other, as were worm length and number of eggs *in utero* (although it was not possible to estimate standard errors for correlations between these two traits). However, the worm number traits were always negatively correlated with worm length and fecundity. The standard errors of the correlations were often large, reflecting the quantity of data available and the heritabilities of the constituent traits.

Correlations between the indicator traits at the final measurement point and 24 week FEC are shown in Table 2.7. Strong negative genetic correlations were observed between faecal egg count and IgA activity and eosinophilia. Fructosamine concentration appears to be moderately positively genetically correlated with faecal egg count. A weak negative correlation was observed between pepsinogen and faecal egg count. An important phenomenon observed here is that the genetic correlations are all opposite in sign to the environmental correlations, resulting in relatively low phenotypic correlations.

Correlations between each of the indicator traits and the necropsy traits are shown in Tables 2.8 to 2.12. In each of these tables, correlations between pairs of traits are not presented when the standard error of the genetic correlation exceeded 0.50.

Table 2.8 illustrates the correlations between faecal egg count, at 24 weeks of age, and necropsy traits. Faecal egg count has a positive genetic

correlation with both the number of adult worms and the fecundity and size of these worms, although the phenotypic correlations are slightly weaker. The strong positive correlation of FEC with worm burden was unexpected given the low heritability of worm burden, but similar strong positive correlations were obtained with the number of adult males (0.62) and the number of adult females (0.70). However, FEC is uncorrelated with the number of L5 larvae.

Correlations between pepsinogen concentrations and necropsy traits are shown in Table 2.9. Worm length appears to be moderately negatively genetically correlated with pepsinogen concentrations, as is the number of eggs *in utero*. The number of L5 larvae is moderately positively genetically correlated with pepsinogen, although the standard error of the estimates is large. Genetic correlations with the number of males (0.28) and females (0.11) were consistent with the worm burden result.

IgA correlations with necropsy traits at 24 weeks are shown in table 2.10. IgA activity appears to be strongly negatively correlated with both worm length and fecundity. Here again, the phenomenon of the difference in sign between genetic and environmental correlations, resulting in low phenotypic correlations, can be observed.

Fructosamine and necropsy trait correlations are shown in Table 2.11. Fructosamine concentration appears to be uncorrelated with worm burden, strongly positively correlated to worm length yet only moderately correlated with worm the number of eggs *in utero*. The previously described difference in sign is also evident in these results. Fructosamine concentration exhibits a strong negative genetic correlation with the number of L4 larvae but not with the number of L5 larvae.

Correlations between eosinophil counts and necropsy traits are shown in table 2.12. Eosinophil count appears to be strongly negatively genetically correlated with worm length, worm burden and the number of eggs *in utero*. Correlations between eosinophil count and numbers of adult males (-0.73) and females (-0.49) are consistent with the worm burden result. The environmental correlations shown here are very low.

2.4. Discussion

This study has enabled us to reexamine the heritability of parasitic and indicator traits, and allowed us to quantify relationships between parasitic and indicator traits, at the genetic, phenotypic and environmental levels. As a broad summary, strong genetic correlations were observed between parasitic and indicator traits, but the corresponding phenotypic correlations were usually weak. However, it must be recognised that many of the genetic and environmental correlations had large standard errors, indicating that they may change substantially if measured in independent populations. For this reason, the discussion will concentrate on overall patterns of results, rather than individual correlations which might be estimated imprecisely.

As previously reported (Stear *et al.,* 1997a), the parasite development traits, mean worm length and the correlated trait mean number of eggs *in utero*, were considerably more heritable than the parasite number traits. In contrast, Gauly *et al.* (2002) reported a heritability value (± SE) of 0.54 (± 0.29) for worm burden yet found worm length to be not heritable, in a small study of Rhoen sheep deliberately infected with *Haemonchus contortus.* Gauly *et al.* (2002) also reported heritabilities for FEC at 16 and 20 weeks of age of 0 and 0.35 ± 0.14 for

Rhoen sheep, and 0.07 ± 0.07 and 0.17 ± 0.07 for Merinoland sheep respectively. The differences between these two studies may be due to differences in nematode species (*H. contortus* vs. predominantly *T. circumcincta*), or perhaps the challenge protocol, i.e. deliberate vs. natural infection. However chance effects due simply to experimental imprecision are also possible.

IgA was found to be highly heritable in this study although no other published heritabilities for IgA were found. However, Gauly *et al.* (2002) found Immunoglobulin G (IgG) activity not to be heritable, and Douch *et al.* (1995) reported heritabilities of 0.18 ± 0.05 for IgG specific to *Trichostrongylus colubriformis* and 0.31 ± 0.05 for antibodies specific to *T. circumcincta*. I observed strong negative genetic correlations between both IgA activity and eosinophil counts and the worm development traits. This result suggests that families with high levels of IgA or eosinophils will have shorter less fecund worms. The IgA result is in agreement with previous work that associated IgA activity with the regulation of worm fecundity (Stear *et al.* 1995; Smith *et al.* 1985). Thus, IgA activity and eosinophil count may be useful traits for selection purposes, with the aim being to increase these trait values in order to decrease worm development and fecundity.

In contrast, genetic correlations between worm development traits and fructosamine were positive and moderate to strong. This indicates that families with high fructosamine concentration have long, fecund worms. Low fructosamine concentrations reflect high protein turnover. High levels of parasite infection are unlikely to decrease protein turnover (Stear *et al.* 2003). Therefore low fructosamine concentrations may modify grazing behaviour, and it may be possible to utilise frucosamine concentration as an indicator trait.

Faecal egg count was positively genetically correlated with worm burden, and negatively correlated with eosinophil count and IgA activity. This is in agreement with the previously discussed results and the role of IgA and eosinophil in the regulation of worm fecundity. The similarity between the correlations of IgA activity and eosinophil counts with the worm development traits could be explained by the fact that eosinophils have a high-affinity receptor for IgA and also that IgA and eosinophil responses are both cytokine driven. During the immune response IgA binds to eosinophils to allow targeting of the parasite (Van Egmond et al. 2001).

An interesting and potentially important phenomenon observed in this study was the difference in sign seen between the genetic and environmental correlations for some of the pairs of traits. This difference in sign leads to the observed phenotypic correlations often being very low. Conceptually, the genetic correlation indicates the underlying genetic relationship between traits. This correlation is estimated utilising information from relatives and thus averages and hence reduces non-genetic effects specific to individual animals at any point in time. Conversely, the environmental correlation reflects non-genetic, i.e. environmental circumstances specific to individual animals at that time point that affect both traits. For many of these traits the environmental correlation may be considered, in part, a result of the infection status of the animals, although experimental error may also contribute to the observed outcome. IgA will be used as an example to interpret these results, as the phenomenon of opposing correlations is seen consistently in the IgA results. An interpretation is that as the genetic correlation indicates the underlying genetic control, a negative genetic correlation suggests that the genetic pathways that control the ability to produce IgA are linked to the genetic pathways that control parasite

development and therefore lead to resistance to infection. The positive environmental correlations suggest that after removing the genetic effects, higher levels of IgA are simply an indication of infection status, i.e. more IgA is produced in response to longer worms. The net consequence of these opposing effects is that underlying genetic relationships may often be masked by weak phenotypic correlations. Whilst this phenomenon was not seen with worm burden traits, these traits were under weak genetic control in these data.

This phenomenon of opposing genetic and environmental correlations is not unique to this study. For example, Morris *et al.* (2003) observed contrasting genetic and environmental correlations between faecal egg count and anti-nematode antibodies in cattle, and results reported by Shaw *et al.* (1999) comparing faecal egg count and IgG concentrations in sheep show the same phenomenon.

In conclusion, this study has shown that necropsy and indicator traits are generally moderately to highly heritable. Moreover, parasitic and indicator traits are moderately to strongly genetically correlated, suggesting that the indicator traits do indeed reflect the animal's ability to respond to infection. Traditionally the selection objective has been to increase parasite resistance by using FEC as the indicator trait. The correlations between indicator traits and necropsy traits observed in this study could provide an opportunity to include parasite development traits, such as worm length and eggs *in utero,* as breeding goals within a selection scheme. Thus by using these indicator traits within a selection scheme it would be possible to influence worm development traits to a greater extent than by using FEC alone, and therefore make greater progress towards decreasing the impact and load of infection as well as decreasing FEC and pasture contamination. Many factors, including cost, optimum age for selection

and logistical implications, would need to be investigated before an economically optimum index or selection procedure could be advocated for commercial use.

Table 2.1 Number of parents used per year

Year	No. of Sires	No. of Dams
1	8	99
2	12	115
3	13	150
4	18	139
5	17	124

Table 2.2 *Summary data for necropsy traits (489 observations for all traits)*

Trait	Mean	Minimum	Maximum	Standard Deviation	Mean (transformed‡)	Standard Deviation (transformed‡)
Worm Length (cm)	0.87	0.57	1.22	0.13		
Eggs *in utero*	18.93	1.05	67	13.56	2.68	0.77
Adult Nematodes	3291	0	21900	3293	7.71	0.93
Total No. of Worms†	6073	150	37900	7368	8.16	1.08
No. of L4 Larvae	2593	0	27100	4797	6.22	2.16
No. of L5 Larvae	173	0	7100	655	4.01	1.28
No. of Adult Males	1391	0	7850	1365	7.15	0.95
No. of Adult Females	1905	0	15400	1988	6.84	0.98

†Total No. of Worms is the sum of fourth-stage larvae, fifth-stage larvae and adult *Teladorsagia circumcincta.*

‡See text for description of the transformations.

Table 2.3 *Summary data for indicator traits*

Trait	Month	Age (weeks)	No. of Observations	Mean	Minimum	Maximum	Standard Deviation
IgA activity (% of standard)	Aug	16	772	12	0	138	15
	Sept	20	962	20	0	110	17
	Oct	24	562	14	0	79	12
Eosinophil count (x5.6 cells/µl)	May	4	389	1.57	0.41	4.2	0.75
	June	8	194	1.47	0	4.17	0.77
	July	12	193	1.75	0	4.73	0.79
	Aug	16	391	2.32	0	4.53	0.94
	Sept	20	391	2.39	0.41	5.01	0.78
Fructosamine concentration (µmol/l)	July	12	204	165.21	81	205	20.66
	Aug	16	204	180.8	100	265	25.4
	Sept	20	592	194.4	96	341	40
Pepsinogen activity (% of standard)	Sept	20	767	30.17	0	281.43	30.7
Faecal egg count (eggs/g)	Oct	24	819	354.9	0	3613	462.5

Table 2.4 *Necropsy trait heritabilities*

Trait	h^2	s.e.
Worm Length	0.53	0.17
Worm Burden	0.13	0.10
No. eggs *in utero*	0.50	0.16
No. of Adult Females	0.08	0.09
No. of Adult Males	0.12	0.10
No. of L4 Larvae	0.06	0.09
No. of L5 Larvae	0.12	0.09
Total no. of Worms	0.12	0.10

Table 2.5 *Indicator trait heritabilities*

Trait	Month	Age (weeks)	h^2	s.e.	c^2	s.e.
Fructosamine concentration	July	12	0.10	0.17	0.53	0.10
	Aug	16	0.05	0.14	0.23	0.11
	Sept	20	0.39	0.16	0.21	0.07
IgA activity	Aug	16	0.46	0.13		
	Sept	20	0.67	0.11		
	Oct	24	0.57	0.15		
Eosinophil count	May	4	0.74	0.25		
	June	8	0.63	0.33		
	July	12	0.31	0.25		
	Aug	16	0.43	0.17		
	Sept	20	0.35	0.15		
Pepsinogen activity	Sept	20	0.56	0.16	0.12	0.07
Faecal egg count	Oct	24	0.33	0.15		

Table 2.6 *Environmental (re) phenotypic (rp) and genetic (rg) correlations amongst necropsy traits.*

Traits	re (se)	rp (se)	rg (se)
Worm Length, Worm Burden	-0.01 (0.14)	-0.09 (0.05)	-0.35 (0.41)
Worm Length, No. eggs *in utero*[†]	0.54	0.79	1.00
Worm Length, No. of L4 Larvae	-0.34 (0.11)	-0.43 (0.04)	-0.96 (0.28)
Worm Length, No. of L5 Larvae	-0.29 (0.14)	-0.24 (0.05)	-0.23 (0.37)
Worm Burden, No. eggs *in utero*	-0.11 (0.13)	-0.11 (0.05)	-0.18 (0.44)
Worm Burden, No. of L5 Larvae	0.08 (0.12)	0.26 (0.05)	0.77 (0.27)
No. eggs *in utero*, No. of L5 Larvae	-0.18 (0.12)	-0.21 (0.05)	-0.36 (0.36)

[†] se not estimable

Table 2.7 *Environmental (re), phenotypic (rp) and genetic (rg) correlations between faecal egg count at 24 weeks of age and final indicator trait measurements.*

Traits	re (se)	rp (se)	rg (se)
FEC, IgA	0.30 (0.16)	-0.13 (0.05)	-0.78 (0.18)
FEC, Eosinophil	0.19 (0.14)	-0.36 (0.04)	-0.97 (0.11)
FEC, Pepsinogen	0.08 (0.19)	-0.02 (0.05)	-0.11 (0.20)
FEC, Fructosamine	-0.15 (0.29)	0.10 (0.06)	0.26 (0.21)

Table 2.8 *Environmental (re), phenotypic (rp) and genetic (rg) correlations between faecal egg count at 24 weeks of age and necropsy traits.*

Traits	re (se)	rp (se)	rg (se)
FEC, Worm Length	0.09 (0.16)	0.19 (0.05)	0.32 (0.25)
FEC, Worm Burden	0.15 (0.10)	0.25 (0.05)	0.65 (0.28)
FEC, No. eggs *in utero*	0.11 (0.16)	0.15 (0.05)	0.21 (0.26)
FEC, No. of L5 Larvae	0.02 (0.10)	0.02 (0.05)	-0.01 (0.36)

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Table 2.9 *Environmental (re), phenotypic (rp) and genetic (rg) correlations between pepsinogen activity at 20 weeks of age and necropsy traits.*

Traits	re (se)	rp (se)	rg (se)
Pepsinogen,Worm Length	-0.09 (0.23)	-0.30 (0.05)	-0.48 (0.21)
Pepsinogen, Worm Burden	0.09 (0.17)	0.09 (0.05)	0.17 (0.38)
Pepsinogen, No. eggs *in utero*	-0.30 (0.22)	-0.26 (0.05)	-0.26 (0.22)
Pepsinogen, No. of L5 Larvae	-0.10 (0.17)	0.08 (0.05)	0.43 (0.34)

Table 2.10 *Environmental (re), phenotypic (rp) and genetic (rg) correlations between Immunoglobulin A activity at 24 weeks of age and necropsy traits.*

Traits	re (se)	rp (se)	rg (se)
IgA, Worm Length	0.27 (0.27)	-0.15 (0.07)	-0.53 (0.24)
IgA, No. eggs *in utero*	0.42 (0.26)	-0.07 (0.07)	-0.62 (0.26)
IgA, Worm Burden	0.03 (0.14)	-0.06 (0.06)	-0.36 (0.46)
IgA, No. of L5 Larvae	0.07 (0.15)	0.00 (0.07)	-0.18 (0.44)

Table 2.11 *Environmental (re), phenotypic (rp) and genetic (rg) correlations between fructosamine concentration at 20 weeks of age and necropsy traits.*

Traits	re (se)	rp (se)	rg (se)
Fructosamine, Worm Length	-0.27 (0.24)	0.17 (0.06)	0.67 (0.27)
Fructosamine, Worm Burden	-0.12 (0.19)	-0.06 (0.06)	0.03 (0.32)
Fructosamine, No. eggs *in utero*	-0.19 (0.37)	0.14 (0.04)	0.31 (0.24)
Fructosamine, No. of L4 Larvae	0.23 (0.30)	-0.12 (0.06)	-0.80 (0.46)
Fructosamine, No. of L5 Larvae	-0.07 (0.25)	-0.07 (0.06)	-0.14 (0.36)

Table 2.12: *Environmental (re), phenotypic (rp) and genetic (rg) correlations between eosinophil count at 24 weeks of age and necropsy traits.*

Trait	re (se)	rp (se)	rg (se)
Eosinophil,Worm Length	-0.05 (0.21)	-0.28 (0.07)	-0.58 (0.27)
Eosinophil, Worm Burden	0.09 (0.13)	-0.04 (0.07)	-0.61 (0.50)
Eosinophil, No. eggs *in utero*	-0.05 (0.18)	-0.29 (0.06)	-0.69 (0.27)

Chapter 3 Development of genetic relationships between indicator traits and measures of *Teladorsagia circumcincta* resistance in Scottish Blackface lambs.

3.1 Introduction

The previous chapter explored the suitability of immunological indicator traits as selection traits for a selective breeding strategy. An implicit assumption of the selection process is that genetic parameters and genetic relationships between traits, particularly heritabilities and genetic correlations, do not change markedly over time. This chapter aims to explore that assumption.

The study reported in this chapter was designed to investigate longitudinal changes in the genetic control of immunological parameters associated with parasitic infection, and their relationship with observed parasite burdens, by means of a quantitative genetic analysis. Parasitic traits obtained from necropsy data and indicator traits that reflect either the local immune response or a pathological consequence of infection were measured, as described in Chapter 2. Patterns of change across time in heritabilities and genetic correlations between traits were then observed. By analysing changes in the relationships between these traits over time I hope to gain an insight into the development of the genetic control of host immune response to parasitic nematodes.

3.2 Materials and Methods

3.2.1 Animals and Husbandry Procedures

Approximately 1000 Scottish Blackface lambs, predominantly twins, were studied over a five-year period (1992-6). The animals were kept on a commercial farm in southwest Strathclyde, Scotland. The animals were bred from 38 sires and 505 dams, some of which were used across years. Single-sire mating was used and consequently the sire and dam of all progeny was

recorded at birth. All husbandry procedures followed standard commercial practice. All lambs were born outside during the final two weeks of April and the first week of May and were continuously exposed to natural mixed nematode infections whilst grazing. Anthelmintic treatment (albendazole sulphoxide) was administered, at the dosage rate recommended by the manufacturer, every 28 days from 4 to 20 weeks of age. Blood and faecal samples were collected every 28 days from 4 to 24 weeks of age, immediately prior to anthelmintic treatment, except that blood samples were not collected in October 1992, October 1993 and August 1995. Approximately half of each cohort was necropsied at 26 or 27 weeks of age in 1992-1995.

3.2.2 Faecal Egg Count:

Faecal egg count, the number of eggs per gram of faeces, was used as the indicator trait to assess the level of infection at each time point, from 4 to 24 weeks. Faecal egg counts were made from a 3 g sample of faeces using the modified McMaster technique (Gordon and Whitlock, 1939; Bairden 1991). Each egg count represented 50 eggs per g. In 1993 duplicate aliquots were counted and in 1994 and 1995 quadruplicate samples were counted. A full description of the analysis of a subset of this dataset was provided by Bishop *et al.* (1996).

3.2.3 Fructosamine Concentrations:

Fructosamine concentration in plasma samples was measured using a Cobas Mira discrete biochemical analyser with a commercial kit (Unimate fructosamine) and calibrated with a specific glycated polylysine calibrant, all of which were obtained from the same supplier (Roche Diagnostics Ltd.). Fructosamine concentrations were measured in July, August and September

1992, September 1993 and September 1994. Further details have been provided by Stear *et al.* (2001).

3.2.4 Eosinophil Counts:

To estimate the concentration of eosinophils in peripheral blood, 10 µl of whole blood was added to 90 µl of Carpentier's solution (Dawkins *et al.* 1989), left for 5 minutes and duplicate samples counted on a haemocytometer. Each cell counted represented 5.6 cells / µl of whole blood. Eosinophil concentrations were measured in May, June, July, August and September 1993 and August and September 1994. Further details have been provided by Stear *et al.* (2002).

3.2.5 Immunoglobulin A activity:

The activity of plasma Immunoglobulin A (IgA) against a somatic extract of 4th-stage larvae from *T. circumcincta* was measured by indirect ELISA, as described by Strain *et al.* (2002). Relative IgA activity was measured as: (observed – standard) / (high control – standard), where the observed value is the sample mean from 3 replicates for the animal, the standard is the mean of 3 replicates from a pooled sample of helminth-naive lambs, and the high control is the mean of 3 replicates from a pool of high-responder lambs (Sinski *et al.* 1995). The pool of high responder lambs was created by combining equal quantities of plasma from 6 lambs that gave strong IgA responses following natural infection. The value for each animal was therefore expressed as a proportion of a positive control. IgA levels were measured in August and September and October of each year with the exception of October 1992 and 1993 and August 1995.

3.2.6 Parasitological assessments:

At slaughter the abomasum was removed, opened along the greater curvature and washed with tap water under moderate pressure. The contents and washings were made up to 2l, from which ten 4ml aliquots were examined to estimate the size of the adult nematode population, i.e. a 0.02 sub sample of the abomasum. The mucosa from one half of the washed abomasum was digested with pepsin-HCl for 6 hours at 42°C; the digest was then made up to 2l to estimate the number of larvae (Armour, Jarrett and Jennings, 1966). Worm length was observed by collecting and measuring a sample of at least 25 female worms from each animal, the number of eggs *in utero* was also counted for these females. The parasite traits recorded were worm length, number of eggs *in utero*, adult worm burden, number of L4 larvae and number of L5 larvae. These traits will be referred to as necropsy traits. Necropsy data was collected from 531 individuals but complete records were only available for 489 individuals.

3.2.7 Data Analysis

The distribution of each trait was assessed; all traits except worm length and fructosamine concentration were skewed and were loge transformed prior to further analysis. The transformations performed were: a straightforward log transformation for eggs in utero; ln (trait + x) where x = half of the measurement increment for worm burden and faecal egg count; and ln (trait + 1) for eosinophil count and IgA activity.

A restricted maximum likelihood package, ASREML (Gilmour et al. 1996), was used to perform univariate analyses with an animal model. The pedigree contained 1613 animals, including 38 sires and 505 dams. In the animal model

the fixed effects fitted were year of birth, field grazed before weaning, sex, day of birth (fitted as a continuous effect) and type of birth (twin or single), along with significant two-way interactions. Measurements of the same variable taken at different time points were analysed as separate traits. Heritability estimates were then obtained for each trait accounting for all known pedigree relationships (Lynch and Walsh 1998). Bivariate analyses were also performed using ASREML to calculate phenotypic and genetic correlations between traits describing parasite burden and traits describing host response to infection. In all cases, an animal model was fitted with the same fixed effects as above. Specifically, bivariate analyses were performed with one trait (worm length, mean number of eggs in utero, worm burden or FEC) at a fixed time point (necropsy), and the other trait (eosinophil count, IgA activity, fructosamine concentration and FEC) at a variable time point. Best-fit lines for phenotypic and genetic correlations are shown in each figure.

These bivariate analyses revealed near-linear changes in genetic correlations as the animal aged (see Results). This development in the host-parasite interactions was then investigated by weighted regression analyses performed on the genetic (or phenotypic) correlations obtained from different time points and trait combinations. The dependent variable in each analysis was the genetic (or phenotypic) correlation, and the independent variables were age (A), and the interaction of age with the parasite (P) and indicator (I) traits. The following regression model was applied:

$$Yijk = bAk + bi(AI)ik + bj(AP)ij + bij(AIP)ijk$$

Where Yijk is the correlation at the kth time point between the ith indicator trait and the jth parasite trait; and b, bi, bj and bij represent the overall time trend (regression) for the correlation coefficients, and the time trends

specific to the ith indicator trait, the jth parasite trait and the ijth combination. The weighting factor for each observed value was the inverse of the standard error of the correlation. All correlations with fructosamine concentration were of the opposite sign to those with other indicator traits (see results), therefore for the regression analysis correlations with fructosamine were multiplied by minus one.

3.3 Results

As described in more detail in Chapter 2, the mean worm length ranged from 0.57cm to 1.22cm with a mean length of 0.87cm. Mean number of eggs *in utero* was 18.93 eggs with a range of 1.05 - 67. Adult worm burden ranged from 0 to 21900, and the mean worm burden was 3291. Worm length and the mean number of eggs *in utero* were found to be highly heritable but worm burden did not exhibit a strong heritability, with values (± SE) of 0.53 ± 0.17, 0.50 ± 0.16 and 0.13 ± 0.10 respectively.

IgA activity and eosinophil count were moderately to highly heritable at all ages. Heritabilities for eosinophil count were 0.74 ± 0.25, 0.63 ± 0.31, 0.31 ± 0.25, 0.43 ± 0.17 and 0.35 ± 0.15 at 1, 2, 3, 4 and 5 months of age, and heritabilities for IgA activity were 0.46 ± 0.13, 0.67 ± 0.11 and 0.57 ± 0.15 at 4, 5 and 6 months. Fructosamine concentrations were initially lowly heritable but then rose, being 0.10 ± 0.16, 0.05 ± 0.14 and 0.39 ± 0.16 at 3, 4 and 5 months. However, at 3 and 4 months there were large litter effects, 0.53 ± 0.10 and 0.23 ± 0.11, which may also contain genetic components. The heritability of FEC rose steadily with age, reaching 0.33 at 6 months of age.

The main focus of this chapter is the genetic correlations between indicator and necropsy traits. These genetic correlations changed markedly and consistently over time, but changes in phenotypic correlations were more

modest. The correlations observed between eosinophil count and the parasitic traits are shown in figure 3.1a-d, along with the regression line of the correlation on time. Genetic correlations with worm burden were imprecisely estimated, simply because the heritability of worm burden was very low and the dataset was not large to compensate for this. Consequently, genetic correlations with worm burden were unreliable for all trait combinations and they are not presented. For all parasite traits, correlations with eosinophil count were initially positive and moderate to strong, and then declined dramatically eventually becoming moderate to strong and negative at 5 months of age.

Correlations involving IgA activity showed a similar trend to those with eosinophil count, despite being estimated over a shorter age range, and are illustrated in figures 3.2a-d. As with eosinophil count, genetic correlations for IgA activity became increasingly negative with time. Genetic correlations with fructosamine concentration showed the opposite trend to those with eosinophil count and IgA activity, generally starting as negative and then becoming more positive with time (Figures 3.3a-c).

The significance of the changes in the genetic correlations across time is shown in Table 3.1, along with the actual regression coefficients. The overall regression of genetic correlation on age was highly significant ($p < 0.001$), indicating that the correlations on average become more negative by approximately 0.2 units per month. The regression models fitting the age by indicator trait and age by parasite trait interactions were also highly significant ($p < 0.001$), indicating that the rate of change does depend on the indicator trait, when considering all parasite traits, or on the parasite trait when considering all indicator traits. For example, the rate of change of correlations with IgA activity is double that of correlations with fructosamine concentration. The indicator trait

by parasite trait interaction was not significant, indicating that general conclusions can be drawn about correlations, across categories of traits. The standard errors of the correlations showed no time trend; rather they reflected the quantity of data available and the heritabilities of the constituent traits.

Lastly, the internal consistency of the correlations was checked, to ensure that the observed time trends in the genetic correlations are not simply a statistical artefact. The correlations were estimated from a series of bivariate analyses, and when comparing across several traits this can produce correlations that are inconsistent with each other, i.e. outside statistical limits. For example, when considering IgA and the number of eggs *in utero*, is it actually possible for IgA activity at 5 and 6 months to show such different correlations with eggs *in utero*, given that IgA activity at 5 and 6 months are likely to be strongly genetically correlated (estimated correlation = 0.92) This is checked by matrix algebra, testing that the determinant of the correlation matrix of these three traits is greater than zero (Searle, 1966). I found that three trait correlations were consistent with each other, i.e. they are statistically plausible.

3.4 Discussion

The major finding in this chapter is that genetic correlations between indicator and parasitological traits showed consistent, marked and significant trends over time. In general they changed from being either weak or counterintuitive, in the case of eosinophil count, to being strong and generally in line with *a prior* expectation at 5 or 6 months of age. I am not aware of other studies that have studied the longitudinal development of traits describing the genetic control of host response to parasites, hence I believe this to be a unique observation. It should be noted that these trends were observed at the genetic

and not the phenotypic level, although it most cases the phenotypic correlations showed a similar but markedly reduced trend. Phenotypic correlations include genetic components, but they also include environmental factors, measurement imprecision and random chance effects, all of which will tend to dilute the observed relationships. Conceptually, genetic correlations are calculated at the family mean level, hence such effects tend to be averaged out.

The changes observed in the genetic correlations for eosinophil count and IgA activity with the necropsy and FEC data were very similar. An explanation of the trend is that as the infection progresses the eosinophil cells become more activated and therefore are more effective at later time points. The eosinophil count and IgA activity are both responses that are driven by cytokines and eosinophils have a high affinity for IgA. During the immune response to parasitic challenge, IgA binds to receptors on eosinophils (Van Egmond et al. 2001). Therefore, similar correlations between these traits and parasite traits are expected, and by analogy similar changes in these responses over time would be expected, with the correlation strengthening as the animal matures. In actual fact, the correlations with eosinophil count actually change sign with time, starting positive and then becoming negative. Unfortunately I do not have the data to assess whether this same pattern would have been observed with IgA activity, as my measurements of IgA commence at the time point when correlations with parasite traits are not significantly different from zero. Although it is plausible why the correlations of IgA or eosinophil counts with parasite traits should become stronger with age, it is less obvious why the relationship should be in the opposite direction in very young lambs. Inhibition of the immune response due to circulating maternally derived antibodies is a possibility, however I don't believe this would be manifested in the genetic

correlation, as in this dataset genetic information is overwhelmingly obtained through sire rather than dam genetic relationships.

The fructosamine concentration correlations were opposite in trend to those of eosinophil count and IgA activity, however the nature of the trend was consistent. Fructosamine concentration reflects average glucose and protein concentrations as well as the rate of protein turnover. *T. circumcincta* can cause a relative protein deficiency as well as an increase in protein turnover and Heath and Connan (1991) observed a decrease in fructosamine concentration following deliberate gastrointestinal infection. However, this is a phenotypic consequence of infection, and my results show that when fructosamine is used as a genetic predictor, the sign of the relationship changes, at least in 5-month old lambs when the fructosamine measurement is adjacent to the parasite trait measurement. The interpretation of this relationship is also confounded by relationships at the phenotypic level, in which the concentration of fructosamine may be driven by worm number (Stear *et al.* 2001a) whereas that of eosinophil and IgA is driven by worm length and worm fecundity (Stear *et al.* 1995; 2002; Strain *et al.* 2002). Once again, however, the reasons for the abrupt changes in the genetic correlations with time are not obvious.

These time-dependent changes in the relationships between indicator and parasite traits have major implications in selective breeding schemes, if one aims to use indicator traits to identify and select lambs that are genetically more resistant to nematode infections. Clearly the objective is to breed sheep that have worm burdens that comprise fewer and smaller worms, and result in a reduced contamination of pasture by nematode eggs. My results suggest that if selection is based on measurements taken at 5 to 6 months of age then combinations of the indicator traits described here should be effective. But, if the

measurements are taken in younger lambs, then selection may be ineffective or even counterproductive. Thus, the age at which selection is made is critical, and the results presented here suggest that measurements should be delayed for as long as possible.

This result also has possible implications for parasite population dynamics. A consequence of the observed time trends is that natural selection of hosts will not result in strong immune responses in lambs of all ages. This will ensure that animal populations will always contain hosts with a range of susceptibilities, thus favouring parasite survival.

In conclusion this study has shown that the genetic basis of host-parasite interactions changes as lambs mature. This result has several implications; it directly affects measurement and selection strategies when breeding sheep for enhanced resistance and it potentially impacts upon parasite population dynamics. However, the mechanisms underlying this phenomenon remain unclear. Elucidation of these mechanisms would require further experimentation.

Table 3.1 Significance of regression[1] of genetic correlations on age.

Trait	Age	Age by Indicator trait	Age by Parasite trait
All traits	-0.217 ***	***	***
IgA		-0.439 ***	
Eosinophil		-0.337 ***	
Fructosamine		-0.242	
Faecal Egg Count			-0.234 ***
Faecal Egg Count 6			-0.237
Worm Length			-0.189
Eggs *in utero*			-0.178

*** ($p < 0.001$)

1. Units of regression coefficients are: change in genetic correlation units per month

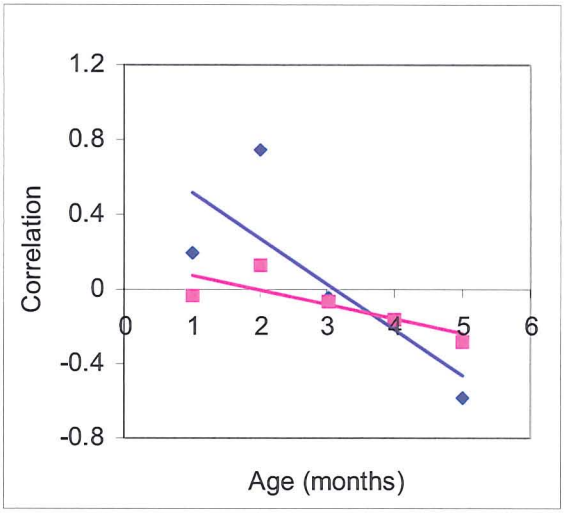


Figure 3.1a: Genetic (♦) and phenotypic (■) correlations between eosinophil count and worm length.

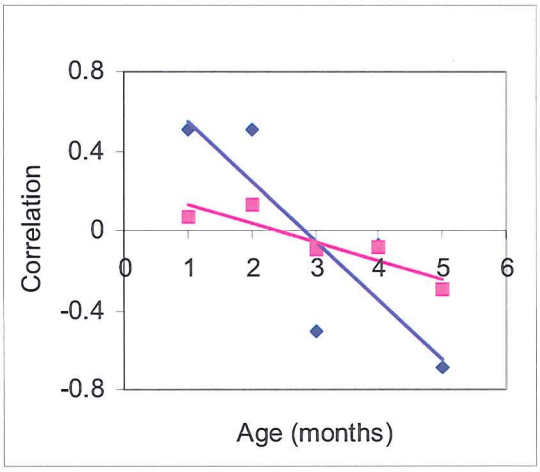


Figure 3.1b: Genetic (♦) and phenotypic (■) correlations between eosinophil count and mean number of eggs *in utero*.

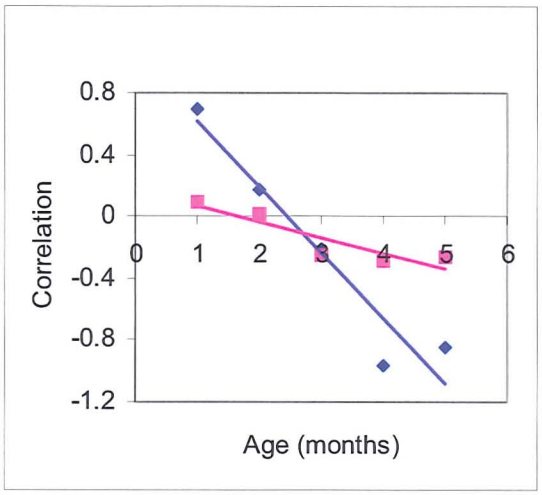


Figure 3.1c: Genetic (♦) and phenotypic (■) correlations between eosinophil count and FEC at the corresponding time point.

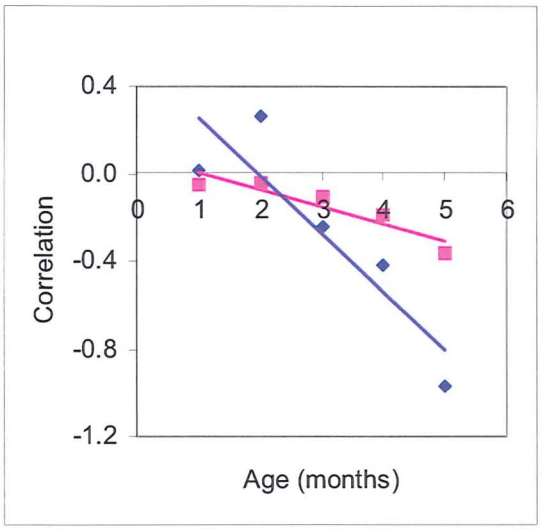


Figure 3.1d: Genetic (♦) and phenotypic (■) Correlations between eosinophil Count and FEC prior to necropsy.

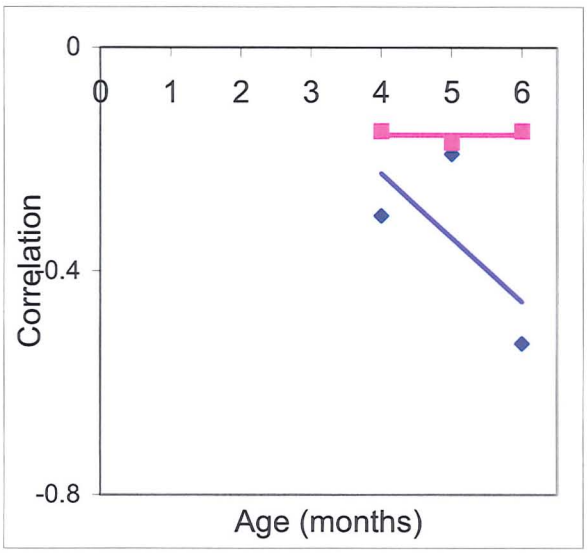


Figure 3.2a: Genetic (♦) and phenotypic (■) correlations between IgA activity and worm length.

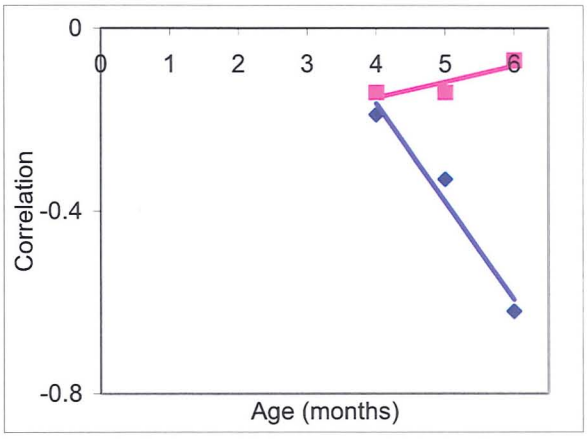


Figure 3.2b: Genetic (♦) and phenotypic (■) correlations between IgA activity and mean number of eggs *in utero*.

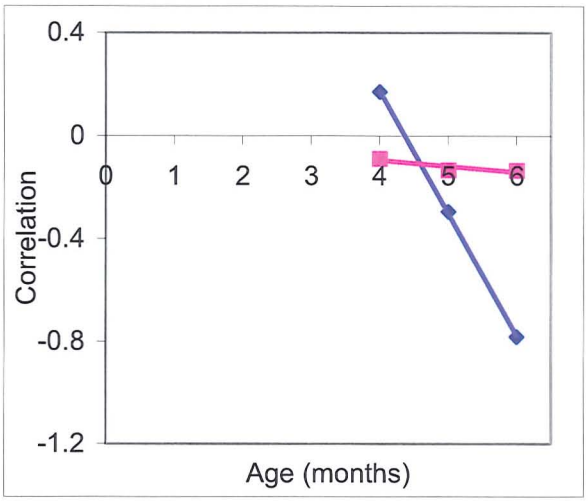


Figure 3.2c: Genetic (♦) and phenotypic (■) correlations between IgA activity and FEC at the corresponding time point.

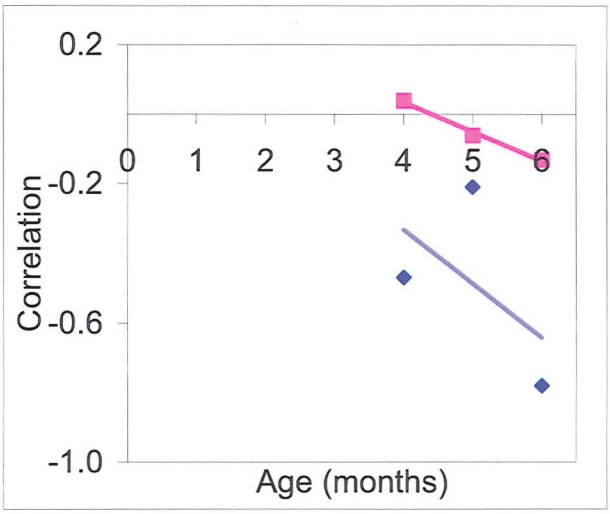


Figure 3.2d: Genetic (♦) and phenotypic(■) correlations between IgA activity and FEC prior to necropsy.

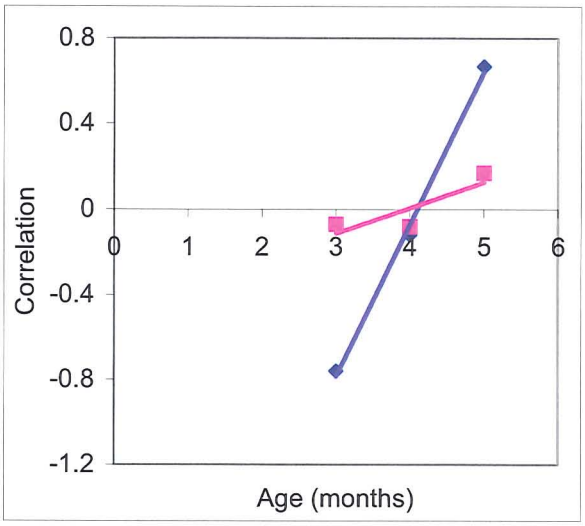


Figure 3.3a: Genetic (♦) and phenotypic (■) correlations between fructosamine concentration and worm length.

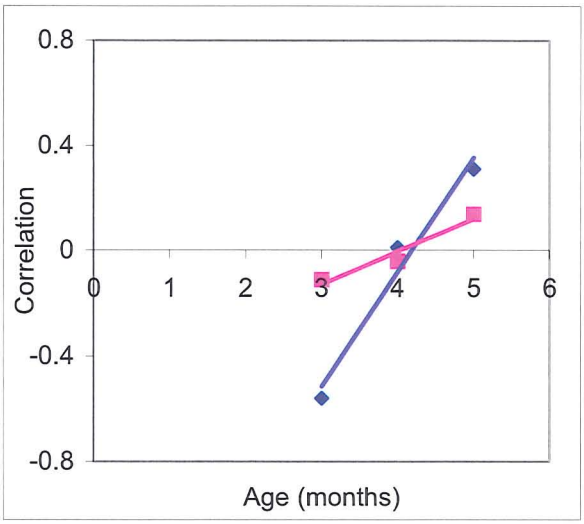


Figure 3.3b: Genetic (♦) and phenotypic (■) correlations between fructosamine concentration and mean number of eggs *in utero*.

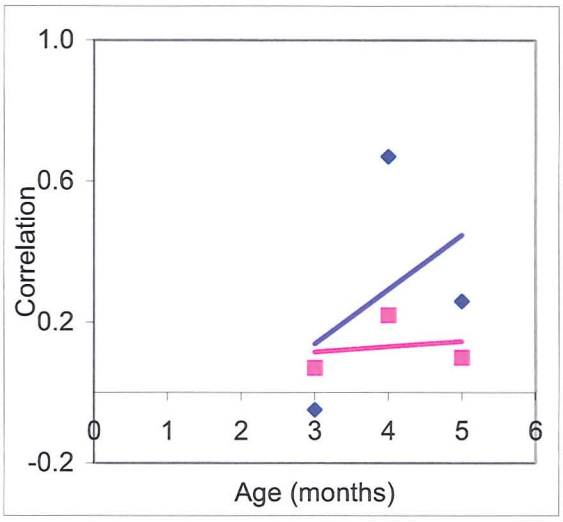


Figure 3.3c: Genetic (♦) and phenotypic (■) correlations between fructosamine concentration and FEC prior to necropsy.

Chapter 4 Quantitative Trait Loci Associated with Parasitic Infection in Scottish Blackface Sheep

4.1 Introduction

Host resistance to parasites varies among individuals and much of this variation is under genetic control (Bishop and Stear, 2003; Quinnell et al 2003; Stear et al 1997a,b; Woolaston and Windon 2001). The previous chapters have provided evidence that supports this assertion.

Collecting and quantifying an indicator trait such as faecal egg count is a costly and time consuming process and also requires the animal to undergo parasitic challenge. Therefore, it would be very useful if it were possible to select directly for parasite resistance, for example by using quantitative trait loci (QTL) in a marker assisted selection scheme. Several studies have reported QTL associated with nematode resistance (Beh et al 2002; Diez-Tascon et al 2002; Janssen et al 2002; Coltman et al 2001; Schwaiger et al 1995). These studies have utilised diverse analysis approaches involving a variety of sheep breeds and nematode species; as a result little overall consensus has emerged.

This chapter aims to identify QTL associated with response to parasitic infection, segregating in a Scottish Blackface flock. In this study FEC and Immunoglobulin A (IgA) activity will be measured. Results presented in previous chapters have suggested that these traits are indicative of host resistance and response to infection. Therefore, the objective of this chapter is to identify QTL for indicators of nematode parasite resistance segregating in a Scottish Blackface flock which has not been subject to any selection on resistance traits.

4.2 Materials and Methods

4.2.1 Animals

The study population comprised 789 Scottish Blackface lambs, comprising 9 half-sib families ranging from 23-141 individuals. The animals were

bred over a 3-year period (2001 – 2003) and sire and dam were recorded at birth for all animals. The complete pedigree for this population contained 4847 animals with records dating back to 1986.

The lambs were born outside and were continually exposed to natural mixed nematode infection by grazing. Lambs were kept in two groups each year with group representing the field grazed. Husbandry procedures followed standard commercial practice. Anthelmintic treatment was administered at the dosage rate recommended by the manufacturer every 28 days from 12 – 20 weeks of age. Treatment was by ivermectin (Oramec drench, Merial Animal Health) in 2001 and 2003 or levimasole (Nilverm, Schering-Plough Animal Health) in 2002.

4.2.2 Phenotypic Measurements

Faecal samples were collected from the rectum of the lamb at 16, 20 and 24 weeks of age. Faecal egg counts were made from a 3g sample of faeces using the modified McMaster technique (Gordon and Whitlock, 1939; Bairden 1991). Four replicates of each faecal sample were counted and each egg represented 12.5 eggs per gram of faeces. Eggs were classified according to whether they were *Nematodirus* spp. or other nematodes which in this study could include the following genera: *Oesophagostomum*, *Chabertia, Bunostomum*, *Trichostrongylus*, *Cooperia*, *Ostertagia*, *Teladorsagia* and *Haemonchus.* Collectively, these are referred to as 'Strongyles' in this paper. A previous study suggested that *T. circumcincta* is the predominant nematode species in this environment, accounting for three quarters of GI nematodes in Scottish sheep (Stear et al 1998).

The activity of plasma Immunoglobulin A (IgA) against a somatic extract of 3rd-stage larvae from *T. circumcincta* was measured by indirect ELISA, as described by Strain *et al.* (2002) on blood samples collected at 24 weeks of age. Relative IgA activity was measured as: (observed – standard) / (high control – standard), where the observed value is the sample mean from 3 replicates for the animal, the standard is the mean of 3 replicates from a pooled sample of helminth-naive lambs and the high control is the mean of 3 replicates from a pool of high-responder lambs (Sinski *et al.* 1995). The pool of high responder lambs was created by combining equal quantities of plasma from 6 lambs that gave strong IgA responses following natural infection. The value for each animal was therefore expressed as a proportion of a positive control.

4.2.3 Genotyping and Map construction

All animals were genotyped using microsatellite markers across regions of varying length on chromosomes 1, 2, 3, 5, 14, 18, 20 and 21. These 8 chromosomes were chosen because previous studies suggested the existence of QTL for nematode resistance (chromosomes 3 and 20) or lamb performance traits such as growth rate or meat quality. Each region contained between 9 and 34 markers. All sires were genotyped for all markers across each region. Offspring were subsequently genotyped for markers that were heterozygous in their sire. In total 139 markers were genotyped. Relative marker locations were established by creating a linkage map for each chromosome using Cri-map (Green et al 1990).

4.2.4 Data Analysis

Data analysis began with an assessment of the distribution of the traits. All traits were transformed prior to further analysis; FEC measurements were log transformed, *ln*(trait + x), where x is a constant used to avoid zero values. Typically x = half the measurement increment for the trait. IgA data was transformed using a cubed root transformation. These transformations successfully reduced the skewness of these traits, resulting in approximately normally distributed data.

For the QTL analysis the traits analysed were IgA, FEC at weeks 16, 20 and 24 for both Nematodirus and other species (i.e. 'Strongyles') as well as an average animal effect. A restricted maximum likelihood algorithm, ASREML (Gilmour et al., 1996) fitting a repeatability model (i.e. ignoring genetic effects), was used to create an average effect for each animal, i.e. the average weighted FEC across the three time points. Fixed effects fitted in this model were year, management group, sex, type (twin or single) and day of birth (fitted as continuous effect), and the calculations were performed on transformed FEC data.

Heritabilities were also estimated using ASREML. An animal model, including all known pedigree relationships (4847 animals), was fitted, with the same fixed effects as above. This analysis was repeated fitting a litter effect (c^2); the significance of the litter effect was tested using a likelihood ratio test.

4.2.5 Estimation of QTL Position

QTL analyses were performed using regression techniques (Knott et al. 1996) implemented by QTL express (Seaton et al. 2002). The probability of

inheriting a particular sire chromosome at a particular position was calculated for each offspring at 1cM intervals. Phenotypes were then regressed upon the conditional probability that a particular haplotype is inherited from the sire, along each chromosome, fitting fixed effects of year, sex, litter size, management group and day of birth (fitted as a covariate). For each regression an F-ratio of the full model including the inheritance probability versus the same model without the inheritance probability was calculated across families; the location of the QTL was indicated by the largest F-value.

4.2.6 Significance Thresholds

The 5% chromosome-wide threshold was determined for each chromosome by permutation testing (1000 permutations) (Churchill and Doerge, 1994). A 5% genome–wide threshold was then obtained by applying the Bonferroni correction (Knott et al. 1998): $P_{genome\text{-}wide} = 1 - (1 - P_{chromosome\text{-}wide})^{n}$, where n is the number of chromosomes. The genome-wide threshold is based on the assumption that by chance you would expect 0.05 significant results per genome analysis.

4.2.7 Confidence Intervals

For each QTL estimate that was significant at the 5% chromosome-wide level confidence intervals were calculated using the bootstrap method (Visscher et al, 1996). 1000 samples with replacement were used to estimate 95% confidence intervals.

4.2.8 QTL Effects

The proportion of phenotypic variance explained by the QTL was calculated as $4(1- MS_{full} / MS_{reduced})$, where MS is the residual mean square from

the regression analysis (Knott et al. 1996). By dividing this phenotypic value by the heritability, estimated using ASREML, this results in the proportion of genetic variance explained by the QTL. As these results came from a half-sib analysis it was necessary to adjust the genetic proportion (GP) value to account for the proportional reduction in phenotypic variance expressed within sire families: Adjusted GP = $GP(1 - h^2 / 4)$. The resulting value estimates the proportion of total additive genetic variance that is explained by the QTL.

4.3 Results

Summary statistics for the FEC traits and IgA are shown in Table 4.1. It is apparent from this data that the Nematodirus eggs are a small proportion of the total egg counts, as expected from previous results (Stear et al 1998). Nematodirus FEC ranged from 0 to 1888 and other species (i.e. 'Strongyles') FEC ranged from 0 to 5325. FEC for both Nematodirus and other species were considerably larger in August than in either September or October. IgA activity against 3rd-stage larvae ranged from 0 to 1.24 with a standard deviation of 0.19.

Heritability estimates for all traits are shown in Table 4.2. The FEC heritability estimates are somewhat variable, being highest in August. IgA appears quite lowly heritable in this study population, although there was also a significant maternal effect.

Significant QTL are shown in Table 4.3. The QTL analysis suggested the existence of QTL on chromosomes 2, 3, 14 and 20, associated with both types of FEC and IgA.

There were several QTL observed that were associated with Nematodirus FEC traits. A QTL associated with Nematodirus FEC September

was observed on chromosome 2 (Table 4.3). The estimated position was 134 cM and this QTL was significant at the 5% genome wide significance threshold. The QTL accounted for 52% of the total additive genetic variation (Table 4.4). A QTL associated with Nematodirus FEC August was found on chromosome 3 at 174 cM. This was significant at both the 5% chromosome-wide threshold and the 5% genome wide threshold (Table 4.3), and it accounted for 26% of the additive genetic variation (Table 4.4).

There was evidence for QTL associated with Nematodirus FEC on chromosome 14. These QTL were associated with average animal effect, FEC August and FEC October at positions 103 cM, 100 cM and 104 cM respectively (Figure 4.2). This appears to be the same QTL effect that is being observed across these traits. All of the QTL observed on this chromosome were significant at the 5% genome-wide level. The QTL for average animal effect also reached significance at 1% chromosome-wide threshold. The QTL accounted for 79%, 40% and 71% of the additive genetic variance in average effect, August FEC and October FEC respectively (Table 4.4). The QTL contour plots shown in Figure 4.2 illustrate the strength of the effects and the agreement across traits.

There was evidence for QTL associated with non-Nematodirus spp. (i.e. 'Strongyles') FEC traits on chromosomes 3 and 20. A QTL associated with the October FEC was found at 150 cM on chromosome 3. The QTL accounted for 37% of the additive genetic variance for the average animal effect (Table 4.4). On chromosome 20 a QTL was observed at 10 cM (Figure 4.3) associated with average animal effect. This QTL was in the region of the MHC and accounted for nearly a third (31%) of the total additive genetic variance.

On chromosomes 3 and 20 QTL associated with IgA activity were also found. The QTL on chromosome 3 was at position 118 cM (Figure 4.1) and was

significant at the 5% genome wide threshold (Table 4.3). The QTL explained 41% of the additive genetic variation (Table 4.4). On chromosome 20 indications of a QTL were observed for IgA activity at 40 cM (Figure 4.3). This QTL was significant at the 5% chromosome-wide threshold (Table 4.3) and the QTL accounted for slightly over half (51%, Table 4.4) of the total additive genetic variation in IgA activity. This QTL is also in the region of the MHC.

4.4 Discussion

This study has identified QTL on 4 chromosomes for various FEC traits and IgA activity. The QTL identified on two of these chromosomes are close to regions that influence immune function.

The QTL on chromosome 3 associated with IgA activity is very close to the Interferon gamma locus (IFNG). IFN-γ has an important role in the regulation of the immune response to pathogens (Urban *et al.* 1996; Wakelin 1996). IFN-γ is a cytokine that is secreted by Th1 lymphocytes. It activates macrophages which then become more capable of killing intracellular pathogens and display increased ability to present antigens. Previous evidence for QTL associated with parasitic infection on chromosome 3 in the region of IFNG has been reported in several studies. Paterson et al (2001) suggested a QTL in the interval IFNG – BMS1617 for a multispecies parasite challenge in Romney divergent selection lines. Evidence for a QTL associated with *T. circumcincta* was reported in Soay sheep again close to IFNG (Coltman et al 2001) and a QTL for *Trichostrongylus colubriformis* was observed in Merino divergent selection lines in the IFNG region (Beh et al. 2002). Although these QTL are very close to the QTL identified in this study they are in diverse breeds, challenged with different species of nematodes.

In sheep the Major Histocompatibility Complex (MHC) is found in 2 regions of chromosome 20. The QTL found on chromosome 20 in this study are both very close to the MHC regions. These regions are likely to contain possible candidate genes as the MHC consists of a group of closely linked genes involved in antigen presentation to the vertebrate immune system. The primary immunological function of MHC molecules is to bind and 'present' antigenic peptides on the surfaces of cells for recognition by the antigen-specific T-cell receptors of lymphocytes. Several studies including Charon et al. (2002), Paterson et al. (1998), and Buitkamp et al. (2002) have implicated both regions of the MHC as QTL for nematode resistance. Schwaiger et al (1995) reported a QTL close to DRB1 in Scottish Blackface sheep facing a *T. circumcincta* natural challenge. Secondly three marker associations within the MHC region were reported in a Roehnschaf flock for haematocrit level (CP73), IgL level (DYMS1) and FEC (BM1815) after an artificial challenge with *Haemonchus contortus* (Janssen et al. 2002). This again is evidence within a similar chromosomal region, despite the fact that the Roehnschaf study involves artificial challenge with a different parasite and different trait measurements.

The QTL effects calculated in this study are very large for some of the traits, particularly when expressed as a proportion of the genetic variance. This may be due to the fact that some of the heritability estimates are quite low, as the QTL effects expressed in relation to the total phenotypic variance are more modest. Additionally, I found the heritability estimates, particularly for Nematodirus FEC, to be very sensitive to the data transformation used, specifically to the increment added to the raw value to avoid zeroes. Therefore, the estimate of the proportion of genetic variability may be less precise than the estimate of the phenotypic variance explained. Additionally, I estimated the

effect of the transformation used on QTL locations and significance values. I found the regression techniques used in QTL mapping to be robust and insensitive to different transformations. In particular, the QTL positions were essentially identical irrespective of the transformation used, and the F ratios were only slightly affected. Thus, the transformation affected the heritabilities but not the QTL estimates, and this may be due to the nature of the data. Nematodirus is not the predominant parasite in the flock used for this study; hence the data is skewed by an abundance of 0 values. Thus, even when transformed the Nematodirus data is not normally distributed and the variance component analysis method used by ASREML is significantly affected by this deviation from normality.

In conclusion this study has provided strong evidence for QTL linked to parasitic infection and immune response on chromosomes 2, 3, 14 and 20. The QTL on chromosomes 2 and 14 affect egg production by Nematodirus spp., and more work is necessary to confirm these associations and identify potential candidate genes. Chromosomes 3 and 20 have been previously associated with nematode resistance and contain candidate genes that influence immune function. Unfortunately there is only a small amount of previously published work relating to QTL for parasite resistance and within those studies there is little common ground regarding breed, parasite challenge and traits measured. The chromosome exhibiting the strongest evidence for a QTL in this study does not have any candidates obvious to us and this requires further work to identify the genes underlying this region. The result of this study suggest that some aspects of parasite resistance are under strong genetic control and with further work this information could be used to select sheep for increased resistance to parasitic infection in a marker assisted selection scheme.

Table 4.1 Summary Statistics

Trait	Age (weeks)	No. of observations	Mean	Max.[a]	Mean of Transformed Data	Standard Deviation of Transformed Data
IgA Activity	24	757	0.13	1.24	0.42	0.21
Nematodirus FEC August	16	740	39.0	1888	3.62	0.74
Nematodirus FEC September	20	722	22.2	675	3.61	0.6
Nematodirus FEC October	24	741	30.3	600	3.67	0.71
Strongyles[b] FEC August	16	740	256	5325	4.85	1.28
Strongyles FEC September	20	721	288	2550	5.19	1.12
Strongyles FEC October	24	741	236	1700	5.12	1.02

[a] The minimum value for each trait was zero

[b] Strongyles refers to all species present other than Nematodirus

Table 4.2 Heritabilities

Trait	h^2	s.e.	c^2	s.e.
Nematodirus FEC August	0.30	0.11		
Nematodirus FEC September	0.21	0.09		
Nematodirus FEC October	0.19	0.09		
Nematodirus Average Animal Effect	0.24	0.09		
Strongyles [a] FEC August	0.50	0.12		
Strongyles FEC September	0.11	0.07		
Strongyles FEC October	0.21	0.09		
Strongyles Average Animal Effect	0.23	0.09		
IgA Activity	0.18	0.09	0.13	0.06

[a] Strongyles refers to all species present other than Nematodirus

Table 4.3 QTL significant at 5% chromosome-wide significance level.

Trait	Chromo-some	Position (cM)	Marker Region	F	5% Chromosome-wide Threshold	5%Genome-wide Threshold	95% Confidence Interval
Nematodirus FEC September	2	134	BM81124-CP79	3.06	2.88	2.96	44 – 203
IgA Activity	3	118	KD103-LYZ	2.48	2.48	2.96	36 – 189.5
Nematodirus FEC August	3	174	BM6433-BMS772	3.43	3.41	2.96	0 – 202.5
Strongyles FEC October	3	150	CSRD111-TEXAN15	2.59	2.44	2.96	0 - 205
Nematodirus Average Animal Effect	14	103	ILSTS002-LSCV30	5.26	2.42	2.96	65 – 123
Nematodirus FEC August	14	100	BMS833-ILSTS002	3.54	3.17	2.96	0 – 151
Nematodirus FEC October	14	104	ILSTS002-LSCV30	3.74	2.61	2.96	32 – 146.5
Strongyles [a] Average Animal Effect	20	10	DYA-MCMA36	2.64	2.44	2.96	0 – 59
IgA Activity	20	40	BM1815-DRB1	2.90	2.45	2.96	1 – 65

[a] Strongyles refers to all species present other than Nematodirus

Table 4.4 Proportions of variation attributable to QTL effect

Trait	Chromosome	Heritability	Phenotypic proportion	Genetic proportion
Nematodirus FEC September	2	0.21	0.12	0.52
IgA Activity	3	0.18	0.08	0.41
Nematodirus FEC August	3	0.30	0.08	0.26
Strongyles [a] FEC October	3	0.21	0.08	0.37
Nematodirus Average Animal Effect	14	0.24	0.20	0.79
Nematodirus FEC August	14	0.30	0.13	0.40
Nematodirus FEC October	14	0.19	0.14	0.71
Strongyles Average Animal Effect	20	0.23	0.08	0.31
IgA Activity	20	0.18	0.10	0.51

[a] Strongyles refers to all species present other than Nematodirus

Figure 4.1 QTL contour plot chromosome 3

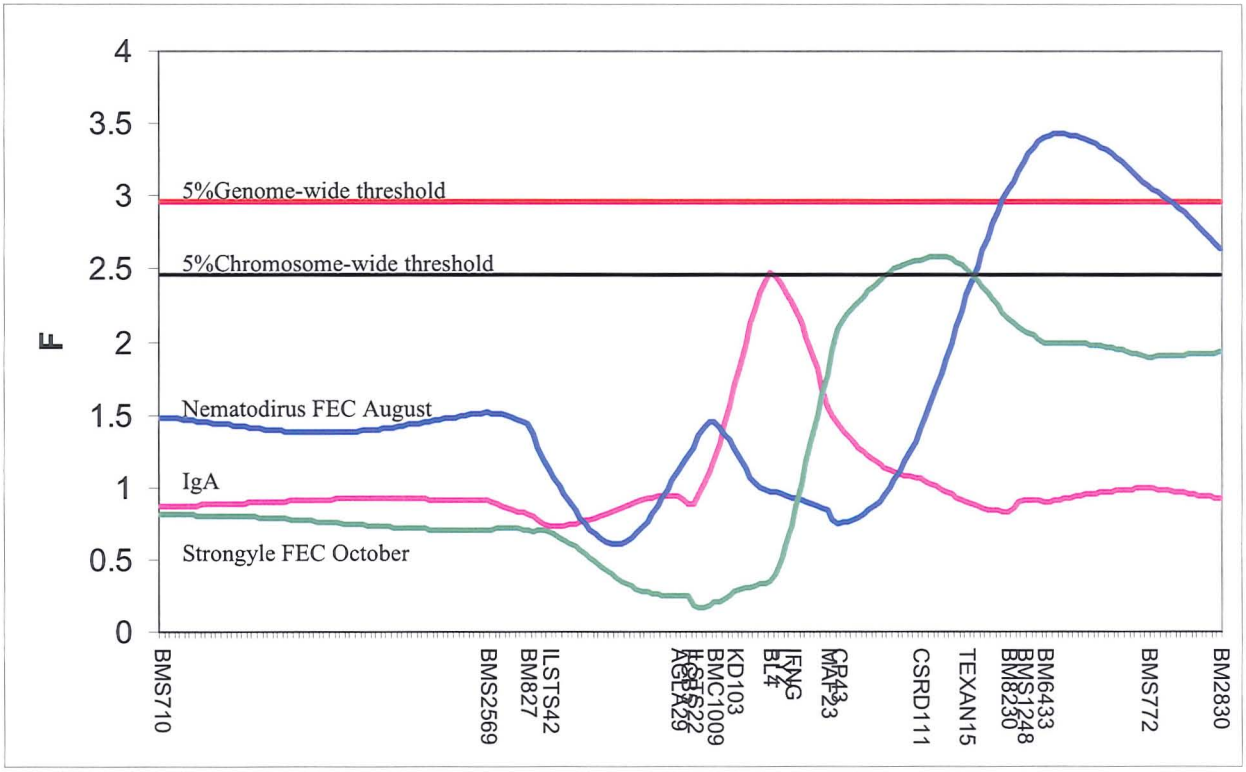


Figure 4.2 QTL contour plot chromosome 14; Nematodirus FEC traits

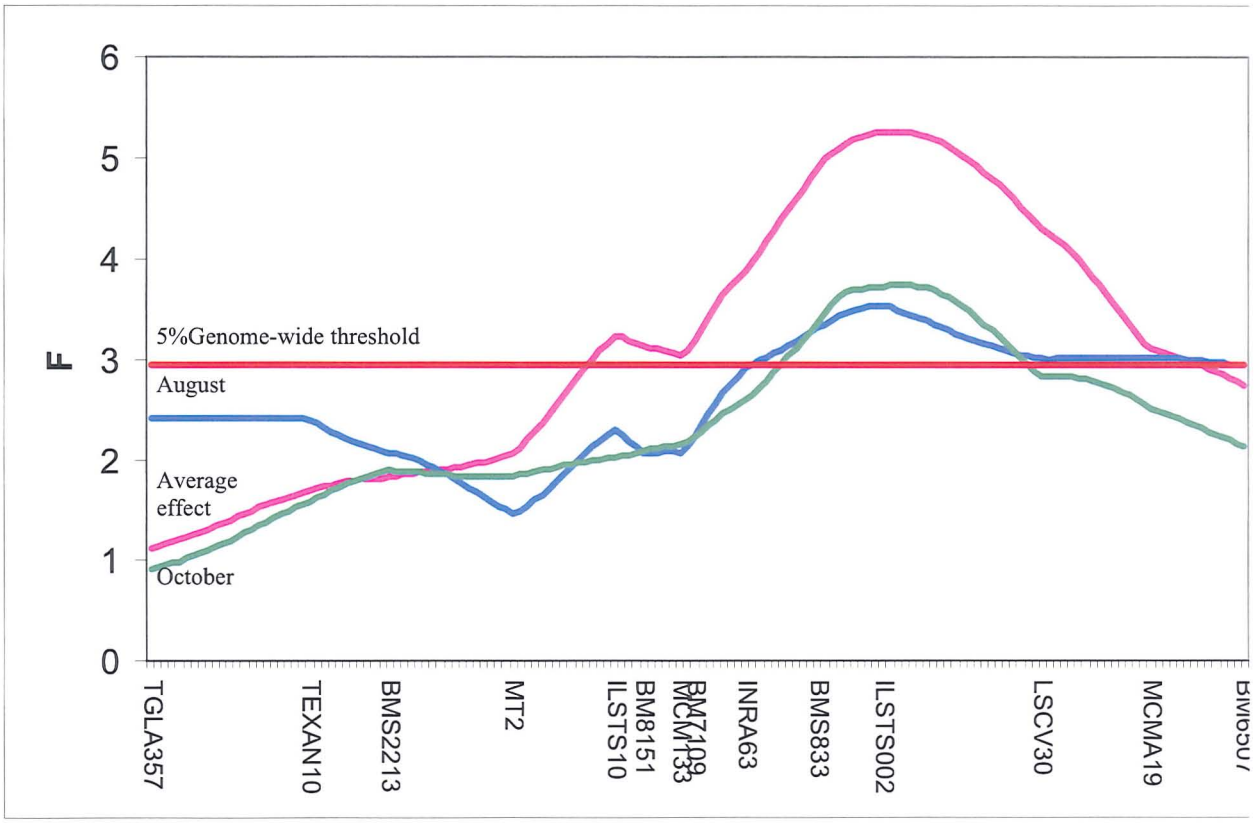
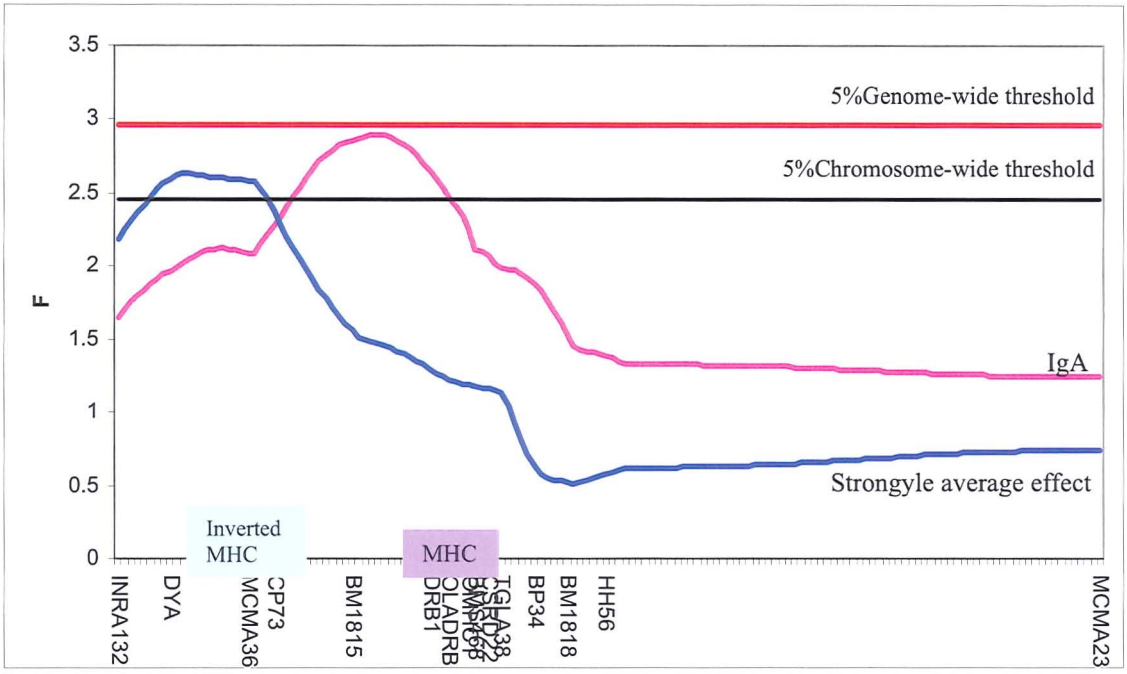


Figure 4.3 QTL contour plot chromosome 20



Chapter 5 Quantitative Trait Loci Associated with Parasitic Infection in a Wide-breed Cross Flock

5.1 Introduction

A possible solution to the gastrointestinal parasite problem is the utilisation of breeds which exhibit increased parasite resistance. Gulf Coast Native sheep are indigenous to the southeast United States. It has been suggested that Gulf Coast animals show increased resistance to infection with *Haemonchus contortus* (Radhakrishnan et al. 1972; Bahirathan et al. 1996; Miller et al. 1998; Amarante et al. 1999). It has been reported that these Native sheep have consistently lower worm burdens than Suffolk sheep of the same age and gender grazing the same pasture (Bahirathan et al. 1996; Miller et al. 1998). The objective of this chapter is to identify quantitative trait loci, associated with resistance to parasitic infection, segregating in the F_2 progeny of a Gulf Coast Native and Suffolk crossbreed, using faecal egg count (FEC) and blood packed cell volume (PCV) as indicators of infection.

5.2 Materials and Methods

5.2.1 Animals

The study population comprised 227 F_2 animals bred over a 3-year period (1997 – 1999). These animals were bred from 3 F_1 rams and 100 F_1 ewes, the F_1 animals were created by crossbreeding Suffolk and Gulf Coast Native sheep (Li et al. 2001). Each year all lambs were kept on the same pasture with their dams until weaning at 12 weeks of age. Faecal and blood samples were collected at 3 time points. Time point 1 was at weaning (12 weeks of age). At 17 weeks of age all lambs were put out to pasture, and after five weeks at pasture the second round of samples were collected (22 weeks of age). At approximately 30 weeks of age all lambs were deliberately infected with 10000 infective larvae (predominantly *H. contortus*) and 4 weeks post infection

the third set of samples were collected (34 weeks of age). Between sampling times all lambs were treated with anthelmintics using the following protocol: ivermectin (Ivomec, 0.2mg/kg), albendazole (Valbazen, 10 mg/kg) and levamisole (Tramisol, 8 mg/kg).

5.2.2 Phenotypic measurements

Faecal and blood samples were collected on 3 consecutive days at the measurement times described above. Faecal samples were collected from the rectum of the lamb. The modified McMaster technique (Whitlock, 1948) was used to determine FEC which was reported as eggs per gram of faeces. Blood samples were collected by jugular venepuncture and PCV was determined using a microhaematocrit centrifuge. For both FEC and PCV the mean of the counts for the three consecutive days was used for analysis.

5.2.3 Genotyping and map construction

Selected animals were genotyped using microsatellite markers across regions of varying length on eighteen chromosomes; these were chromosomes 1, 3, 4, 5, 6, 8, 9, 10, 11, 13, 15, 16, 18, 19, 20, 21, 23, and 24. Each region contained between 2 and 14 markers. 190 lambs and their parents were genotyped at a total of 63 markers; these lambs were the upper and lower tails (20%) for FEC after natural challenge. Relative marker locations were taken from the Sheep Best Positions Linkage Map Version 4.3 (Australian Sheep Gene Mapping Website, Maddox 2004).

5.2.4 *Data Analysis*

The distributions of all traits were assessed for normality. All FEC traits were transformed prior to further analysis. These traits were subjected to a log transformation ln (trait + x) where x is a constant used to avoid zero values. Typically x = half the measurement increment which in this case was 25. These transformations successfully reduced the skewness of these traits resulting in approximately normally distributed data. The PCV traits did not need to be transformed prior to further analysis.

For the QTL analysis the traits analysed were FEC and PCV at weeks 12, 22 and 34 and an average animal effect that is described below. A restricted maximum likelihood algorithm, ASREML (Gilmour et al., 1996) fitting an animal repeatability model (i.e. ignoring genetic effects), was used to create an average effect for each animal for both FEC and PCV. This effect was an average effect across time as both traits were measured at 3 time points. This animal model also fitted fixed effects: year, sex, type (twin or single) and day of birth (fitted as continuous effect). The average effect was calculated from the transformed FEC data.

5.2.5 *Estimation of QTL Position*

QTL analyses were performed using regression techniques implemented by QTL express (Seaton et al. 2002). The probability of inheriting a particular sire chromosome at a particular position was calculated for each offspring at 1cM intervals (Knott et al. 1996). Phenotypes were then regressed upon the conditional probability that a particular haplotype is inherited from the sire, along each chromosome, fitting fixed effects of year, sex, birth type and day of birth (fitted as a covariate). For each regression an F-ratio of the full model including

the inheritance probability versus the same model without the inheritance probability was calculated across families, the location of the QTL was indicated by the largest F-value.

5.2.6 Significance Thresholds

The 5% chromosome-wide threshold was determined for each chromosome by permutation testing (1000 permutations) (Churchill and Doerge, 1994).

5.2.7 Confidence Intervals

For each QTL estimate that was significant at the 5% chromosome-wide level confidence intervals were calculated using the bootstrap method (Visscher et al, 1996). 1000 samples with replacement were used to estimate 95% confidence intervals.

5.3 Results

FEC values observed in this study ranged from 0 to 149933 with a mean value of 12411. PCV counts also varied from 7.3 to 37.0 with a mean value of 22.2. Phenotypic variances were close to 1 for both natural infections (time points 1 and 2) but somewhat larger for the deliberate infection (time point 3).

Table 5.1 shows the results from the QTL analysis. There is evidence at the 5% chromosome-wide threshold for QTL on 4 chromosomes. Chromosome 1 yielded evidence for a QTL for both FEC and PCV at time point 1 at the same chromosomal position (Figures 5.1 and 5.2). This QTL was significant across all families for FEC but only in family 1 for PCV. FEC 2 was associated with a QTL on chromosome 6. Chromosome 9 yielded strong evidence for a QTL associated with PCV2 with an across family analysis giving a significant F value

at a 5% chromosome–wide threshold. However a within family analysis of family 3 produced an F value significant at the 1% chromosome-wide threshold. Chromosome 19 produced a significant result for FEC average effect and PCV 3 in the same region.

Contour plots are shown only for chromosome 1 (Figures 5.1 and 5.2) as these were the best profiles. Other profiles were not such a good shape possibly because of two reasons, firstly when the QTL appeared at one end of the analysis region and secondly when there were very few markers on the chromosome in question.

5.4 Discussion

The predominant result from these analyses is the apparent difference in the nature of the same trait between time points. The same QTL effects were not observed across time points or between time points and the average animal effect for both FEC and PCV. An example of this is the QTL for FEC Average animal effect of chromosome 19 which was significant at the 5% threshold, but individually none of the three time points were significant. Also for the other QTL reported in this study an effect was seen at an individual time point, but not for the overall average. This is quite surprising as a previous study, described in chapter 4, in a Scottish Blackface flock has shown reasonable levels of concordance between individual time points and average animal effects for Nematodirus FEC.

It appears that the nature of the trait is changing with time. It is then interesting to question which time point provides the best estimate of resistance status. At weaning (time 1), lambs appear to be relatively immune incompetent and after the summer pasture challenge (time 2) immune 'competent', hence the

rationale for time 2 being a better reflection of potential resistance than time 1. Time 2 also represents the infection situation encountered by most grazing sheep, thus is more applicable to sheep production systems. Time 3 involves a one time experimental infection and reflects the response to a large single bolus infection and is therefore not representative of the challenge faced by commercially produced sheep. There are therefore distinct factors that make each time point different. Form a genetic perspective FEC at weaning is somewhat different to FEC several weeks later at pasture, and FEC following artificial challenge may then be a different trait again. From the results of this study it has become apparent that the 3 measurement points are representing very different traits even when the same phenotypic measurements are collected.

The QTL on chromosome 1 for FEC is in a similar region to a QTL reported in Merino divergent selection lines for *Trichostrongylus colubriformis* FEC (Beh et al. 2002). None of the other QTL observed in this study have any similarity in position to previously published work, however QTL studies on parasite resistance are few in number and appear to have utilised diverse analysis approaches involving a variety of sheep breeds and nematode species (Beh et al 2002; Diez-Tascon et al 2002; Janssen et al 2002; Coltman et al 2001; Schwaiger et al 1995). There also appears to be a lack of literature relating QTL to PCV measurements and parasitic infection.

In conclusion the study described in this chapter has provided evidence for QTL associated with parasitic nematode infection on 4 chromosomes, segregating in a flock that has been subject to selection.

Table 5.1 QTL significant at 5% chromosome-wide significance level.

Trait	Family	Chromosome	Position (cM)	Marker Region	F	5% Chromosome-wide Threshold	95% Confidence Interval
FEC1	All	1	264	MAF109 – BMS119	4.73	4.30	223.6 – 332.6
PCV1	1	1	264.6	MAF109 – BMS119	8.73	6.96	218.6 – 332.6
FEC2	All	6	42.6	MCM53 – MCMA14	4.01	3.80	2.6 – 95.6
PCV2	All	9	80.1	CSRD240 – MCM63	3.03	2.95	80 - 93
PCV2	3	9	80.1	CSRD240 – MCM63	13.35*	5.00	80 - 89
FEC Average Effect	All	19	16	BMS517 – CSSM006	4.67	3.57	15 – 44.5
PCV3	1, 3	19	17	BMS517 – CSSM006	4.82	4.34	15 – 61.5

* Significant at 1% chromosome-wide threshold

Figure 5.1: QTL contour plot of chromosome 1 for trait FEC 1

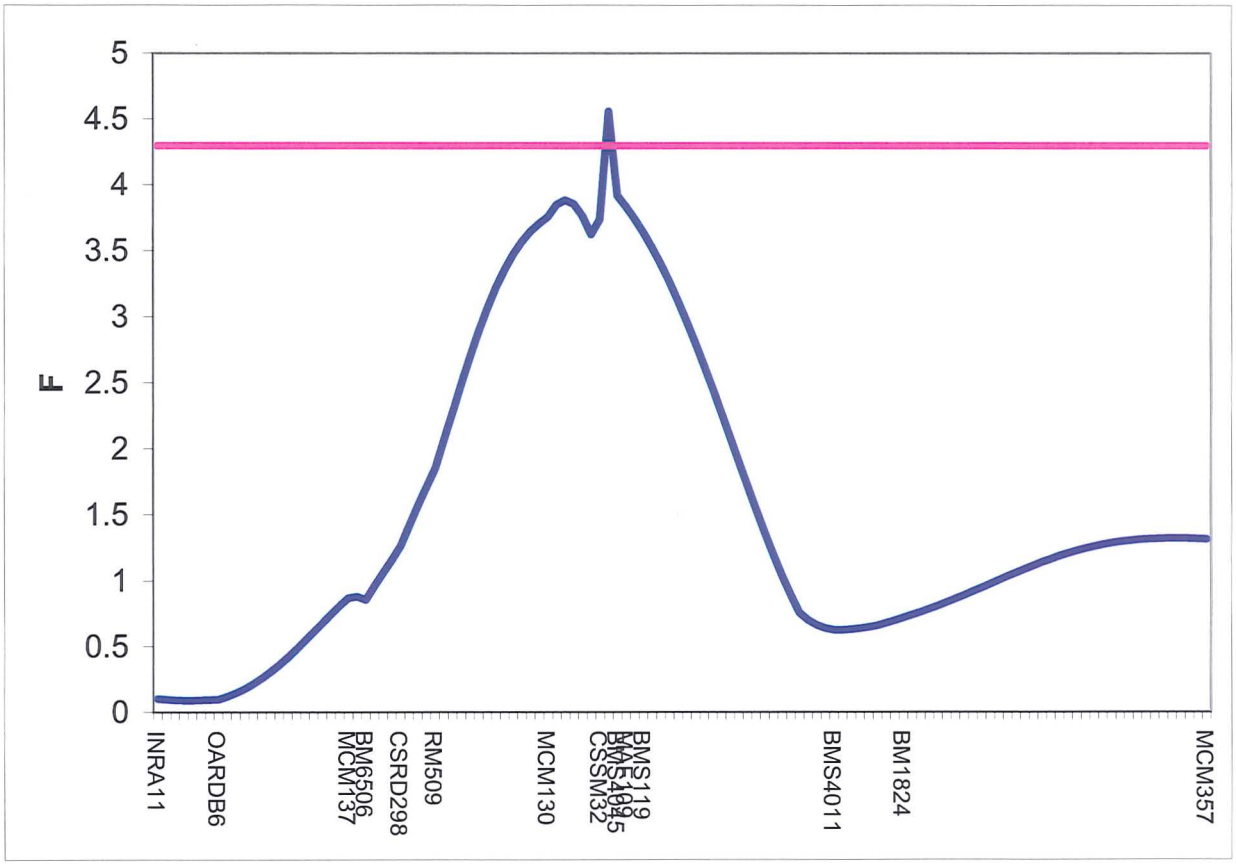
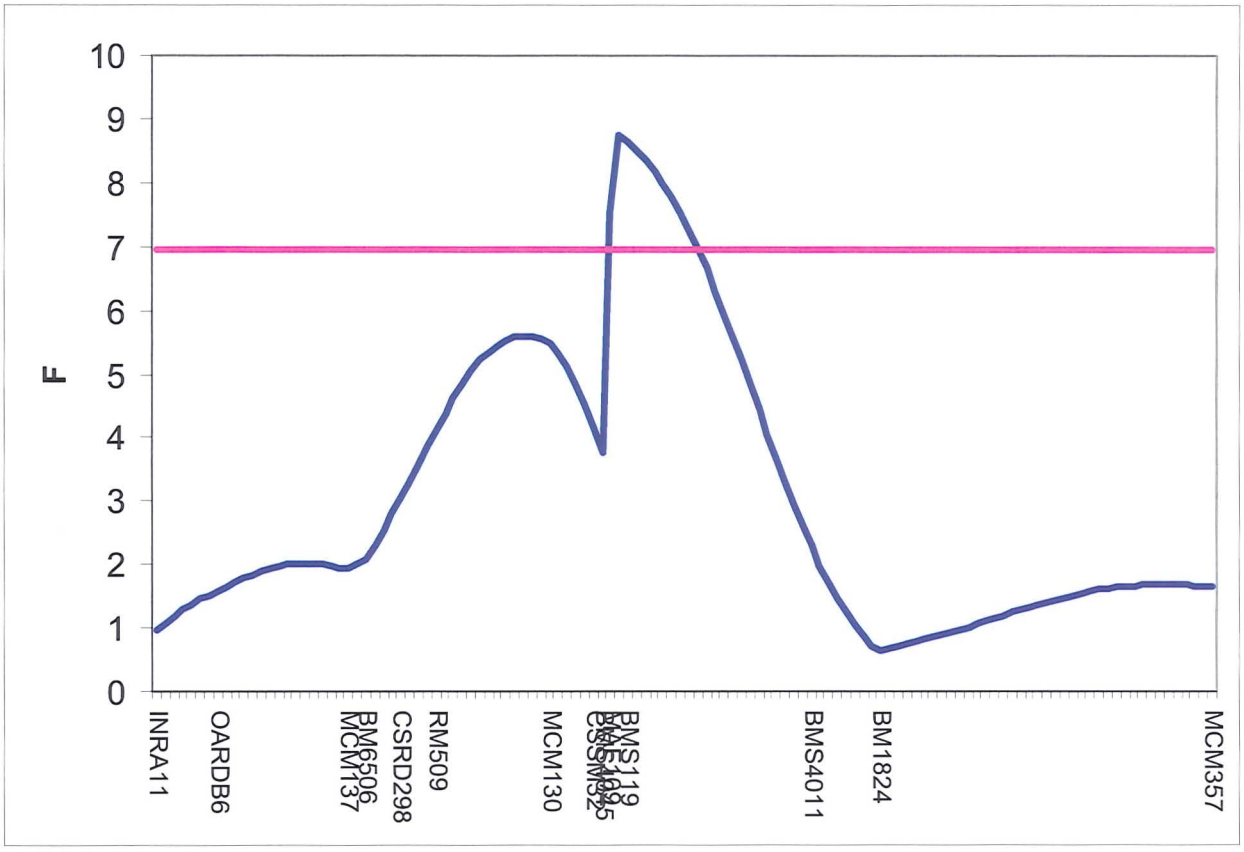


Figure 5.2: QTL contour plot of chromosome 1 for trait PCV 1 within family analysis of family 1



Chapter 6 Parasite species interactions

6.1 Introduction

As concomitant nematode infections are the norm rather than the exception in sheep (Petney and Andrews 1998) it is important to understand interactions between species in order to develop effective management strategies. While *T. circumcincta* is the predominant parasite in UK sheep (Stear et al 1998), other parasite species are also present. In order to implement effective parasite control it would be useful to know if the host animals that are heavily infected with *T. circumcincta* are also more susceptible to infection with other parasite species. Therefore in this chapter I investigated the interactions between different parasite species within the host, using worm burden data collected at necropsy from the Tandlemuir flock. Relationships between FEC and parasite species were also analysed.

6.2 Materials and Methods

6.2.1 Animals

Approximately 1000 Scottish Blackface lambs, predominantly twins, were studied over a five-year period (1992-1996). The data analysed in this chapter was collected from the same animals used in previous chapters. A full description of the animals can be found in Chapters 2 and 3.

6.2.2 Parasitological Assessments

At slaughter (6-7 months of age) the abomasum was removed, opened along the greater curvature and washed with tap water under moderate pressure. The contents and washings were made up to 2l, from which ten 4ml aliquots were examined to estimate the size of the adult nematode population.

The mucosa from one half of the washed abomasum was digested with pepsin-HCl for 6 hours at 42°C; the digest was then made up to 2l and a 0.02 sub sample was used to estimate the number of larvae (Armour, Jarrett and Jennings, 1966). The parasite traits recorded were adult worm burden, number of males and number of females for all nematodes. The parasite species for which these traits were recorded were *Teladorsagia circumcincta*, Nematodirus spp., Cooperia, *Haemonchus contortus*, *Trichostrongylus axei* and *Trichostrongylus vitrinus.* Some species were absent in some years (see table 6.2)

6.2.3 Data Analysis

The first stage of the analysis was to investigate if interspecific interactions were present within the parasite community, using regression analysis. Specifically, the worm burden data were analysed to investigate the effect of the magnitude of the **burden** of one species on that of another species. A separate analysis using binary data, created to reflect the presence or absence of a parasite, was used to investigate the effect of the **presence** of a parasite species on another. Using these techniques all parasite species present were regressed upon each other, with year fitted as a fixed effect. In the binary parasite burden analysis *T. circumcincta* worm burden was fitted as a covariate. All traits were right skewed. Therefore, in order to perform regression analyses on these skewed traits a generalized linear model was used fitting a negative binomial error distribution. Where significant interactions were observed the direction of the effect was observed and classified according to the physical location of the parasite, as upstream (posterior to anterior), downstream

(anterior to posterior) or same locale for parasites occupying the same section of the gastrointestinal tract.

The next stage of the analysis was to consider if there was a genetic component affecting the interactions observed, by investigating the presence of significant sire effects. To identify such effects regression analyses, as described above, were repeated using a generalised linear model fitting sire as a random effect.

Regression analyses were also performed to investigate the relationship between the numbers of adult worms of each species present and FEC. Only two species, *T. circumcincta* and *Cooperia*, were found to have a significant effect on FEC. Following these analyses, functions were then investigated to predict faecal egg count from worm burden data. A gamma type function previously described by Bishop and Stear (2000) was adapted to perform this prediction:

$$\text{Predicted FEC} = e^{a} X_1^{b} e^{cX1}$$

Where X_1 represents the adult worm burden of each species. This was fitted by taking logarithms of both sides of the equations, thus reducing it to a linear equation:

$$ln(\text{FEC}) = a + b\, ln(X_1) + c\,(X_1)$$

However, this equation did not take into account the effect of other parasite species (X_2). Given that two species had shown a significant effect it was essential to include this effect in the FEC prediction, thus the gamma function described above was modified to include two parasite species:

$$\text{Predicted FEC} = e^{a} X_1^{b} e^{cX1} X_2^{d} e^{fX2}$$

This function was also fitted after linearising the equation as described above. Initially the predicted FEC from a particular species was calculated,

evaluated at the mean level of burden for the other species. Following these predictions a joint prediction was calculated.

6.3 Results

Summary statistics for all of the parasite species are shown in Table 6.1. As not all parasite species were present in every year, Table 6.2 illustrates the occurrence of each species by year. Table 6.3 shows the number of sheep with non-zero adult worm counts recorded for each parasite species in each year. It is clear from this table that, as expected, essentially all animals were infected with *T. circumcincta*. Burdens of other species varied between years, and all species, except *T. circumcincta*, exhibited highly skewed distributions due to an abundance of zero values. *H. contortus* and *T. axei* occurred very rarely within the flock studied. *Nematodirus*, *Cooperia,* and *T. vitrinus* were always present in some animals although the number infected varied between years.

The generalised linear model regression analyses yielded many interesting results. These results are shown in Table 6.4, in which significant results are described by classification as upstream or downstream, positive or negative and level of significance. In this table *H. contortus* appears only as an explanatory variate; this is due to this parasite only occurring in one year and within that year it was present in very small numbers, thus this data was not robust enough to be regressed upon as a response variate. No significant sire effects were reported from the regression analyses, indicating that genetic effects on worm burdens were not significantly different from zero.

A positive upstream (posterior to anterior) effect was observed between *T. vitrinus* and *T. circumcincta*. As the converse effect was not significant, this suggests that as the *T. vitrinus* burden increases in the small intestine, then the

T. circumcincta burden increases in the abomasums, but not *vice versa*. A positive downstream (anterior to posterior) effect between *Cooperia* and *T. circumcincta* was observed. Again the converse effect was not significant, thus suggesting that as the *T. circumcincta* burden increases in the abomasum, then the *Cooperia* burden in the small intestine also increases. Some significant results were observed with *H. contortus*. However due to the rare occurrence of *H. contortus* in this study, it was only present in one year, it is possible that these associations are not an accurate representation.

From the regression of parasite species on FEC only *Cooperia* and *T. circumcincta* were found to have significant effects. The function predicting FEC jointly from *T.circumcincta* burden and *Cooperia* burden was:

$$\text{Predicted FEC} = e^{2.623}\,(TC)^{0.3170}\,e^{-0.0001257(TC)}\,(Coop)^{0.0777}\,e^{(Coop\ (0.0001582)}$$

These prediction functions are illustrated graphically in Figures 6.1 and 6.2. The figures show the relationship between predicted FEC and *T. circumcincta* burden at the mean *Cooperia* infection level and the relationship between predicted FEC and *Cooperia* burden at the mean *T. circumcincta* level, respectively, as well as histograms illustrating the actual parasite burdens. The histograms show that the majority of animals have worm burdens of less than 5000 for each species, thus I have more confidence in the predictions on the left-hand side of the graphs, thus coinciding with where more real data is available. As the predictions progress beyond this point, less data is available and thus the predictions may become less accurate. It is also apparent when looking at these figures that there is a density dependence effect for *T. circumcincta*; thus FEC initially increases but then decreases as worm burden increases. However this does not appear to be present for *Cooperia*, where FEC

increases almost linearly with increasing *Cooperia* burden, at least up to worm burdens of ca. 10,000. From regression analysis of these functions it appears that *Cooperia* has a much greater effect on FEC than *T. circumcinta*. This is indicated by R^2 values of 0.56 and 0.32 for *Cooperia* and *T. circumcincta* respectively, when fitting only one species at a time, thus suggesting that *Cooperia* burden 'explains' almost twice the proportion of variation in FEC than *T. circumcincta* burden. This may help to account for the observation that FEC appears to fluctuate between years as it is possibly reflecting the changes in *Cooperia* infection levels more than *T. circumcincta* levels. However this is possibly only true for FEC measured in October and November, as during this time period the FEC means are higher. The dataset does not allow one to meaningfully relate FEC measured at earlier ages to worm burdens.

The final analysis involved calculating the predicted FEC using the equation described above to show FEC as a joint function of *T. circumcincta* and *Cooperia* worm burdens; this is shown in Figure 6.3. However due to the exponential nature of *Cooperia*-predicted FEC, in which predicted FEC becomes very high with high *Cooperia* burdens, the *Cooperia* prediction was truncated at the maximum observed faecal egg count. This is represented by a grey horizontal line in Figure 6.2. This figure visually demonstrates that *Cooperia* burden is having a greater effect on FEC than *T. circumcincta* burden.

6.4 Discussion

This study has provided evidence for parasite species interactions within the host animal. This agrees with a previous analysis of the same data which reported weak but significant positive correlations amongst most species in the numbers present in the host (Stear *et al.* 1998). In both this study and Stear *et*

al. (1998) the interaction between *T. circumcincta* and *T. vitrinus* showed the largest effect. Stear *et al.* (1998) suggests that the positive correlations among species observed in this study may be due to differences in exposure to infection. The distribution of infective larvae on pasture means that a mouthful of grass which contains species 'a' is likely to contain infective larvae of other species. Again in agreement with the results presented in this chapter no competitive interactions were observed between small intestinal species (Stear *et al.* 1998). This may be due to the fact that these species occur at different sites within the intestine (Urquhart *et al.* 1996).

Results involving interactions with *H. contortus* must be treated with a certain amount of caution as this species was only present during one year of the study, and then in small numbers. *H. contortus* is the predominant parasite in southern Europe and is seen only sporadically in Scotland during hot summers. Thus, although a previous study has suggested that *T. circumcincta* interferes with the establishment of *H. contortus* by altering the abomasal environment (Dobson and Barnes 1995), it was not possible to draw conclusions from the results presented here.

The regression result suggesting that *Cooperia* has a greater effect on FEC than *T. circumcincta* is consistent with previously published results (Stear et al. 2005). Stear reported that populations with high mean egg counts did not necessarily have more adult nematodes but had a greater number of adult nematodes from species other than *T. circumcincta*. This could have considerable implications on the effectiveness of selection strategies currently in use that are based on FEC as an indicator of parasitic burden. If FEC is not truly indicative of *T. circumcincta* infection then this may explain why the usefulness of FEC varies between years. This may be further explained, as *Cooperia* is

always present in the flock studied however the prevalence of infection varies between years. Therefore, the fluctuations in FEC that are observed between years may reflect more accurately the changes in *Cooperia* burden not *T. circumcincta* burden.

This relationship between FEC and the burdens of the different worm species needs to be investigated further for two reasons; firstly it is necessary to investigate the underlying genetic relationships involved in controlling these parasitic infections as it is these genetic relationships which will determine the utility of FEC in breeding programmes and, secondly, if FEC is more strongly linked with *Cooperia* than *T. circumcincta* then, as described in earlier chapters, more appropriate indicator traits are available for selection purposes (Davies et al 2005). In chapter 2 indicator traits were described that were linked to the immunological response, and these traits were also found to have strong genetic correlations with worm development traits. Given that the IgA activity measured was for IgA specific to *T. circumcincta* this trait may well provide a more accurate indication of *T. circumcincta* infection. It would be useful to investigate the correlations between parasite species-specific IgA activity and FEC at corresponding time points. This may provide evidence that IgA activity is indeed a more accurate reflection of *T. circumcincta* infection and elucidate the true infection levels represented by FEC. From the work reported in this chapter it appears that FEC alone may not be an optimal indicator trait and it is now maybe the right time to explore more deeply the relationships between FEC and other indicator traits, in order to provide the most effective selection protocols.

Given that management practices utilised at present are largely based on anthelmintic treatment, and due to the resistance exhibited by parasitic nematodes to these compounds making it necessary to find alternative

solutions, the understanding of parasitic interaction becomes essential. The majority of host animals will have a multiparasitic infection and the results from this study suggest that complex interactions occur between these parasites. It is thought that multiparasitic infections can play a significant role in the pathogenic processes occurring in the host. As a result, this can complicate the diagnosis and the prognosis of specific diseases as well as having an effect on the evolution of the host, the parasites and the resulting disease processes (Petney and Andrews, 1998).

One alternative control procedure which has been considered, but as yet is not commercially available, is vaccination. Previously published vaccination work has been against a single parasite species, yet this background of a multiparasite infection will again complicate the situation. Lello et al. (2004) developed a model to investigate the role of inter-specific pathogen interactions on the overall impact of vaccines on gut helminth communities in the rabbit (*Oryctolagus cuniculus*). The Lello results suggest that vaccination against one parasite might produce unexpected and marked increases in the abundance of another previously innocuous parasite species, by releasing it from the negative impact of the targeted parasite. This suggests that within-host interspecific parasite interactions may have a role in determining the overall impact of vaccines on gut parasite communities. In conclusion it appears that when developing such vaccines it is imperative that the background of multi-parasitic infection is considered, as strategies focusing on single parasite species may not take account of the actual infection situation occurring at the herd level.

In conclusion, this study demonstrated that the complexities of multiparastic infection, previously reported in other host species, are present within this commercial sheep flock. This raises important questions regarding

current management procedures and also the development of new control strategies.

In relation to the results presented in earlier chapters the main question has to be are the traits measured actually reflecting the infection / parasite species that we think they are? In chapter 4 are the QTL results for Strongyles FEC representing *T. circumcincta* or *T. circumcincta* and *Cooperia*? We must also raise the question: in a QTL based selection scheme do these interactions matter? As with QTL selection there is no need for previous infection; if the QTL are related to the predominant species and provide some level of control then, are the associations of these QTL with other parasite species problematic?

At present it is not possible to answer these questions but merely speculate on possible effects. There may be effects of selection on such QTL which may alter the inter-specific interactions, yet without further investigation it is not possible to say whether this would be a favourable effect. Perhaps indicator traits linked to immunological response will be less susceptible to such effects than FEC, due to the multi-parasitic infection maybe having a stronger relationship with the immune response than with FEC. Also traits such as antibody activity are highly species specific and given that it can be difficult to differentiate between some species when performing FEC measures, and such measures are often split, for example into Strongyles (which covers multiple species) and *Nematodirus,* perhaps immunological traits provide a more species-specific measure.

However, it is clear that new control strategies must not only utilise traits which truly reflect the parasitic burden, but also take into consideration the entire parasitic range within the host. They must aim to control the infection through

increased immunity to all parasitic infection not just the predominant or highly pathogenic species.

Table 6.1 Summary statistics for all parasitic species present

Trait	No. of obs	Mean	Minimum	Maximum	Standard deviation
T. Circumcincta AM	526	1371	0	7850	1356
T. Circumcincta AF	526	1885	0	15400	1959
T. Circumcincta WB	526	3255	0	21900	3257
Nematodirus AM	526	154	0	1800	277
Nematodirus AF	526	93	0	1400	193
Nematodirus WB	526	246	0	2900	438
Cooperia AM	526	651	0	12400	1453
Cooperia AF	526	807	0	15000	1655
Cooperia WB	526	1458	0	27400	3071
T. vitrinus AM	526	191	0	4100	453
T. vitrinus AF	526	256	0	5800	588
T. vitrinus WB	526	448	0	9600	1009
H. contortus AM	526	1.6	0	400	20.8
H. contortus AF	526	3.3	0	600	35.1
H. contortus WB	526	4.9	0	1000	54.9
T. Axei AM	526	21	0	1700	133
T. Axei AF	526	21	0	1650	131
T. Axei WB	526	42	0	3300	258

Table 6.2 Occurrence of parasite species in each year

Trait	Year 1	Year 2	Year 3	Year 4
T. circumcincta	*	*	*	*
Nematodirus	*	*	*	*
Cooperia	*	*	*	*
T. vitrinus	*	*	*	*
H. contortus				*
T. axei		*	*	*

* Presence of parasite for the year indicated.

Table 6.3 Number of sheep with non-zero worm burdens, for each category of nematode and year

Trait	Year 1	Year 2	Year 3	Year 4
T. circumcincta	109	104	157	156
Nematodirus	70	64	33	86
Cooperia	32	70	93	138
T. vitrinus	63	28	45	107
H. contortus	0	0	0	9
T. axei	0	6	2	29

Table 6.4 Significance of regressions of one parasite species burden on another. Rows represent the explanatory variate and columns the response variate. Descriptions of effects indicate where significant effects were observed: red and green text indicates site of infection as abomasum and small intestine, respectively.

	T. circumcincta	*Nematodirus*	*Cooperia*	*T. vitrinus*	*T. axei*
T. circumcincta			*** + DS		
Nematodirus					
Cooperia					
T. vitrinus	*** + US				
T. axei					
H. contortus		** + DS			

DS Downstream Effect: Occurs when a species which inhabits the anterior gastrointestinal tract is having an effect on a species which inhabits a posterior portion of the tract.

US Upstream Effect: Occurs when a species which inhabits the posterior gastrointestinal tract is having an effect on a species which inhabits an anterior portion of the tract.

+ / - Indicate if the effect is positive or negative.

*, **, *** : Indicate significance level ($p<0.01$, 0.005, 0.001)

Figure 6.1 The relationship between predicted faecal egg count (left y axis) and the number of adult *T. circumcincta* at the mean *Cooperia* burden level. The histogram (right y axis) represents the observed adult *T. circumcincta* burden.

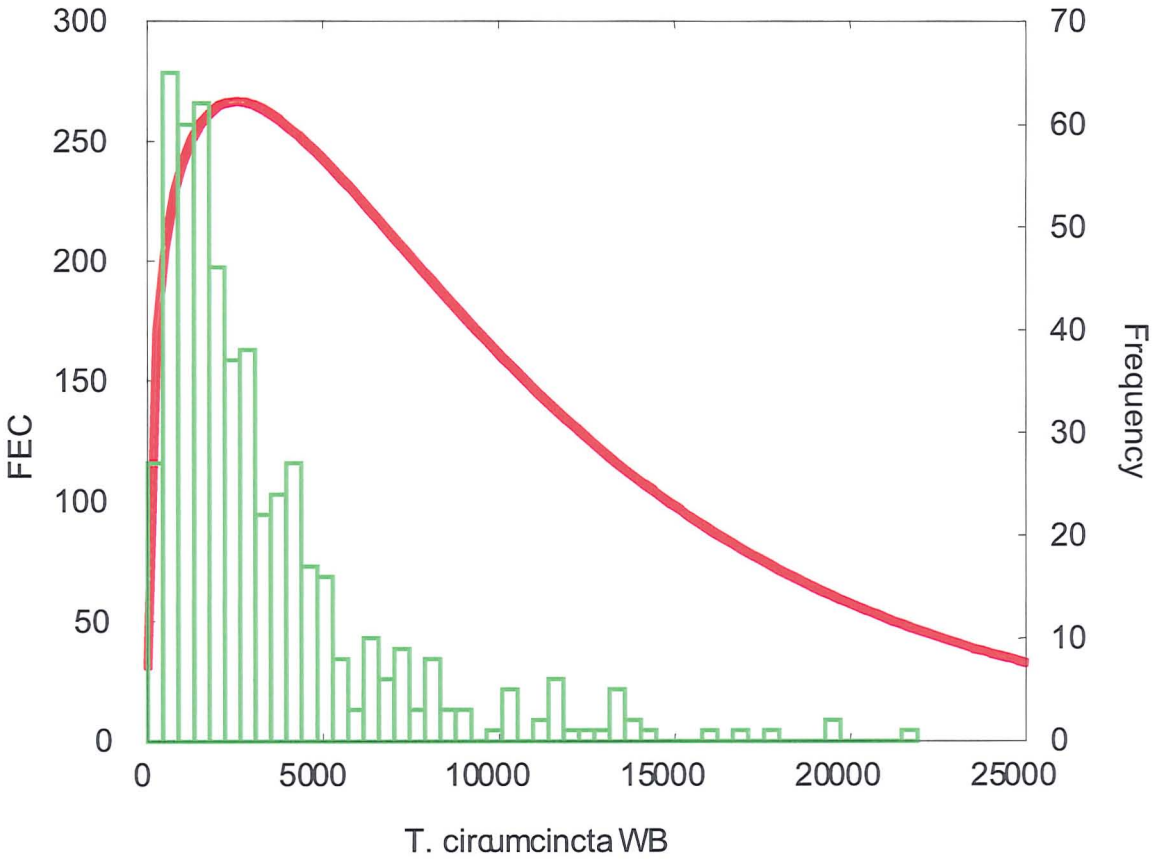


Figure 6.2 The relationship between predicted faecal egg count (left y axis) and the number of adult *Cooperia* at the mean *T. circumcincta* burden level. The histogram (right y axis) represents the observed adult *Cooperia* burden.

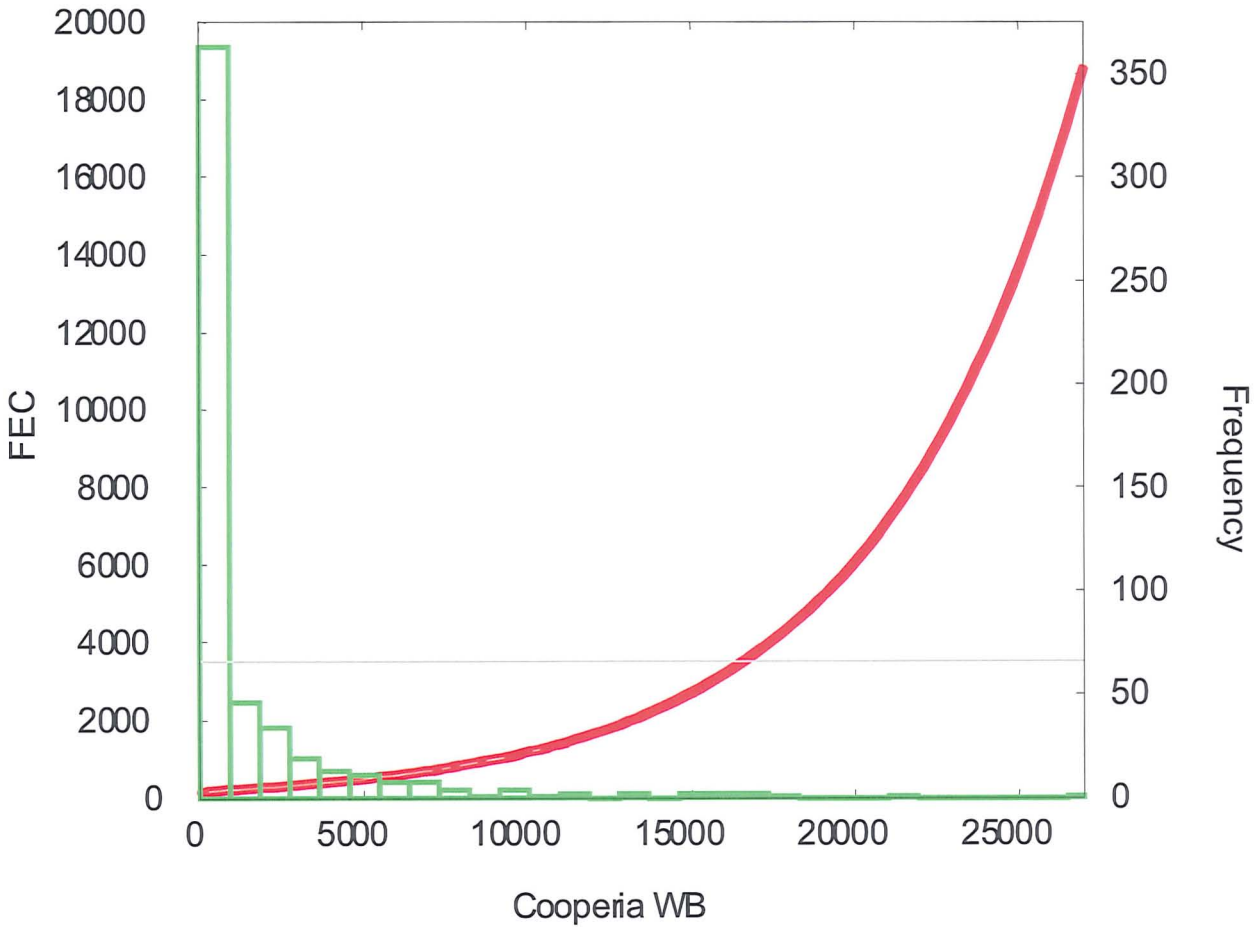
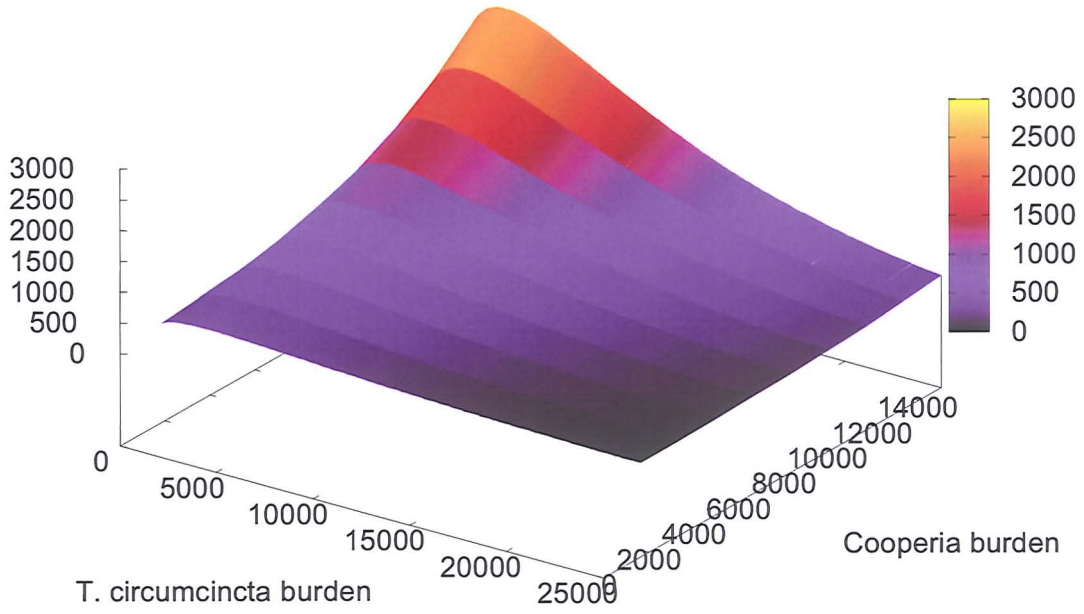


Figure 6.3 FEC (y axis) as a joint function of *T. circumcincta* (x axis)and *Cooperia* (z axis) worm burdens.



Chapter 7 General Discussion

In this chapter the results presented in this thesis will be discussed followed by a discussion of specific issues which have arisen from those results. However firstly I would like to take a step back from these specific issues and consider wider perspectives on disease genetics and how the work described in this thesis fits into this ever-expanding bigger picture.

7.1 Broad Perspectives

Genetic variation in disease resistance has been published for all major livestock species, for all major categories of infectious agent, pathogen or parasite and in all major production systems (Davies, Bishop and Giuffra, 2006; Nicholas, 1987). However at present there are very few disease control strategies which include breeding for resistance implemented within the animal breeding industry. Evidence from both industry and academia suggests that at present for most major livestock diseases there is a gap between research outputs and control strategies needed by industry. By using recent genomic technologies it may be possible to facilitate the identification and exploitation of host genes contributing to resistance, and subsequently to start to bridge the gap between scientific results and practical solutions. The work described in this thesis goes some way to bridge this gap as it provides evidence for alternative indicator traits to FEC which may more accurately reflect the level of infection, thus allowing more appropriate strategic dosing of stock rather than treating the whole flock. Secondly strong evidence for QTL associated with resistance traits was described, these QTL are now under investigation within three major commercial breeds in the UK.

The work described in this thesis has looked at various aspects of the genetics underlying the host response to parasitic infection in sheep. Each

chapter has yielded interesting results which in turn have then raised further questions. Given that parasitic disease costs the UK sheep industry £84 million per year (Nieuwhof and Bishop, 2005) and consumer concern regarding food safety issues is ever increasing, it is now essential that integrated strategies for parasite control are developed and implemented in the near future.

From a wider perspective it is now becoming apparent that integrated disease control strategies are the best solution to disease problems, due to the increasing incidence of drug and vaccine failure. Parasitic infections such as those investigated in this thesis provide a unique opportunity within disease resistance genetics studies. This is due to the fact that the disease is endemic, all of the animals in the flock are exposed to the infection and subsequently develop different intensities of infection, and as this kind of infection is rarely fatal, it allows a wide spectrum of responses to be observed. Therefore, as well as providing insights into the future control strategies which can be developed to control parasitic infection, this study may also be used as a model for diseases which are more difficult to study. By using parasitic disease it allows the longitudinal changes of the host response to be studied over an extended period; this may provide information on the development of such responses which can then be applied to pathogens which cause the animal to deteriorate in a much shorter time frame, thus eliminating the opportunity to observe such changes.

Using parasitic disease in sheep as an example let us now investigate the benefits of breeding for disease resistance. Overall animal health will improve as selection will decrease the intensity of infection which in turn decreases pasture contamination. Ultimately this results in a decreased parasitic challenge and thus increased performance for all animals subsequently grazing

the same pasture (Bishop and Stear, 2003). Therefore this indicates that a large part of the overall result is an epidemiological benefit of genetic improvement, as by increasing resistance of the flock exposure to infection is decreased. This work is also a good example of how it is essential that both the genetic and epidemiological factors and effects are considered if optimal solutions are to be found, when breeding for disease resistance.

There are some examples of livestock disease where genetics has been successfully used in developing control measures, the best example being Marek's disease in chickens. Genetic management of Marek's disease is well established in intensive poultry systems with historic selection on response to infection and specific B alleles within the MHC. However, concurrent vaccination strategies have been associated with more pathogenic strains of the virus, although cause and effect is difficult to establish. Marek's disease is still the subject of many studies and recently several QTL associated with resistance have been identified. Once these QTL are more clearly defined, they could be added to the already established control strategies.

Dermatophilosis provides another successful example of the use of genetics to solve a disease problem. Dermatophilosis is a severe skin infection of tropical cattle which induces a loss in productivity and a 15% mortality rate (Maillard et al., 2002). Strong genetic control has been documented and MHC alleles have been found to be strongly associated with resistance (Maillard et al. 1999, 2002). In the study of Maillard, resistant animals appeared to never become infected, whereas the most susceptible animals exhibited severe clinical signs and died. These results were verified in independent populations and selection removing the most susceptible animals from within a population

reduced the disease prevalence from 0.76 to 0.06 within a 5-year period (Maillard et al., 2002).

However evidence for such host genetic control has not been published for all major diseases. For example in pigs two major endemic diseases, porcine reproductive and respiratory syndrome (PRRS) and post weaning multisystemic wasting syndrome (PMWS), have little published evidence of genetic variation in resistance. These are complex disorders which would benefit greatly from an integrated control strategy, however at present the diseases and associated traits are not clearly defined and thus are somewhat further away from a production level implementation of a genetic-epidemiological solution.

Another disease that has been subject to much study is bovine mastitis. As described earlier, trait definition is an inherent problem in the study of this disease and although selection takes place on conformation traits, somatic cell count and infection history, udder conformation and infection history are lowly heritable and the inclusion of QTL would no doubt greatly improve the selection process. However although many QTL have been identified for several associated traits, at present an optimal integrated approach is yet to be developed.

In comparison with the diseases described above, the evidence presented on the genetics of resistance to parasitic infection in sheep is somewhat in the middle of the spectrum of disease genetics work underway at the present time. Strong evidence for QTL has been described, however major genes are yet to be identified, and traits have been carefully defined in order to provide the basis on which an optimal integrated strategy for disease control may be developed. Given that this work is now being taken further within

commercial flocks, it is the one step closer to providing an industry-level optimal strategy.

7.2 Results

This thesis contains several chapters presenting results, each of which is a complete 'stand-alone' study. However, the underlying theme of the host response to parasitic infection allows the chapters to be linked together to create a bigger picture, even though this research has involved different breeds, traits measured, parasites and methods of infection. This section will summarise the results of each chapter whilst also linking them together to form a greater understanding of the underlying host biology related to parasitic infection.

Chapter 2 involved analysis at a fixed time-point, 6 months of age, and heritabilities and genetic correlations between indicator traits and parasitological (necropsy) traits. The aim was to investigate if the immunological traits measured were heritable and strongly linked to parasitic traits, thus determining their suitability as selection traits for a selective breeding program. This chapter presented results which indicated that the immunological indicator traits and the parasite development traits were moderately to highly heritable whereas the parasite number traits were lowly heritable. In particular IgA activity and eosinophil count were found to be strongly negatively correlated with the parasite development traits, suggesting that families with increased IgA activity would have shorter less fecund worms. This is in agreement with a previous study by Stear et al. (1995b). An interesting phenomenon was observed in this study, in that the environmental and genetic correlations were often opposite in sign, as has been observed previously in both cattle (Morris et. al 2003) and sheep (Shaw et. al 1999). Thus, this chapter concluded that the indicator traits in

question, particularly IgA activity and eosinophil count, were strongly genetically correlated with worm development traits and may be suitable selection criteria to increase flock resistance.

However although the evidence from Chapter 2 suggested that the candidate traits were of good quality, I was not aware of any studies which had reported on the longitudinal development of such traits and thus decided to explore the longitudinal changes in these correlations. The major finding in chapter 3 was that the correlations between indicator and parasite development traits exhibit consistent, marked and significant trends over time. This appears to be a unique observation as no other reported cases were found in published. The strong trends were observed at the genetic level however at the phenotypic level similar but markedly reduced trends were observed. When considering the results of chapters 2 and 3 together, it becomes apparent that these time-dependent changes pose major implications for selective breeding schemes, as they suggest that the age at which selection takes place is critical to the success of the scheme and also that the genetic basis of host-parasite interactions is changing as the lambs mature. Conclusions based on these results suggest that 5-6 months is the optimum age for selection as trait measurements taken in younger lambs may be ineffective and possibly counter-productive. However this is only based on measurements taken up to 6 months and studies over a longer time period may provide new information.

On the basis of Chapters 2 and 3 it appears that although I have determined several fairly robust indicator traits to provide a more accurate description of the infection status of the host, when using indicator traits it is essential that the animal undergoes previous infection in order to trigger the immune response. However if QTL could be identified that are linked to these

indicator traits then marker assisted selection could be implemented which would allow selection without infection. Thus chapter 4 investigated evidence for QTL associated with parasite resistance within an independent Blackface flock. Evidence was reported of QTL mapped to regions of 4 chromosomes, 2, 3, 14 and 20, associated with both FEC traits and IgA activity. Some of these mapped close to regions known to influence immune function, IFNG (chr. 3) and MHC (chr. 20). Several studies have reported QTL associated with parasitic infection close to these regions; IFNG (Paterson et. al, 2001; Coltman et al, 2001; Beh et al, 2002 and Moreno et al, 2006) and MHC (Charon et al, 2002; Outteridge et al. 1996 ; Paterson et al. 1998 and Buitkamp et al 2002). Moreno et al. (2006) also reported QTL on chromosomes 2 and 14. This is the only published evidence other than that presented in this thesis to report a QTL for FEC on chromosome 14. However even though a small amount of literature with similar results has been found there is little common ground regarding breed, parasite challenge and traits measured, thus direct comparisons are impossible at present. Yet the results are promising and are now being investigated in commercial Texel, Charollais and Suffolk flocks.

Chapter 4 involved a purebred Blackface flock which was not selected for increased parasite resistance, thus ensuring a range of responses was observed. Chapter 5 however, again aiming to discover QTL associated with parasite resistance, involved a wide-breed cross flock developed from a resistant breed and a susceptible breed (to *H. contortus*). Although this was a small dataset it still yielded some interesting results with QTL found on chromosomes 1, 6 ,9 and 19, associated with FEC and PCV. Although this provides an interesting comparison with chapter 4, there is no concordance in the results. This is possibly due to several reasons, firstly this is a wide-breed

cross flock involving a tropical breed, secondly it used a challenge from a different parasite species and, finally, the final time-point involved deliberate infection as opposed to all natural infection in the previous chapter. Unlike the results described in chapter 4 where some concordance between measurements taken at differing time points was observed, in the breed cross study different QTL are observed at different time points for the same trait. It appears that the nature of the trait is changing with time, and also that the QTL differ depending on the challenge method. This conclusion was also reported by Moreno et al. (2006).

After 4 chapters investigating the underlying host genetics of parasitic infection, Chapter 6 considers data at the level of the parasitic interactions which go on within the host animal. Although the previous results have been focused on the predominant parasites, *T. circumcincta* and Nematodirus, it is known that an animal can play host to a varied gastrointestinal parasite community, thus chapter 6 aimed to investigate these interactions and their effect, if any, on the indicator traits we wish to implement in selection strategies. The major finding in this chapter was the relationship between FEC and species other than *T. circumcincta*. The strongest association was with Cooperia which exhibited a far stronger influence on FEC than *T. circumcincta*, thus raising the possibility that the predictive ability of FEC as an indicator of *T. circumcincta* worm burden may be somewhat limited. When considering that FEC is the historically utilised indicator trait, maybe it is not giving as true a reflection of *T. circumcincta* worm burden as we would like and thus integrative breeding strategies are now needed in order to control nematode infections in general.

Once put together the chapters of this thesis impart an informative account of the problems and challenges arising in the control of gastrointestinal

parasites, and also point towards possible control measures to be integrated into new management strategies. Specific issues are discussed in the next section.

7.3 Specific Issues

Although each chapter contains its own significant results and these in turn represent a section of the overall 'story', new questions have arisen as the work progressed. Predominantly these issues arise from chapters 3 and 6, simply because these chapters ask more complex questions. Chapters 2, 4 and 5 address straightforward questions relating to direct production issues and produced results such as heritabilities, genetic correlations and evidence for QTL which can be applied to industry level breeding schemes. Chapters 3 and 6 are somewhat more 'experimental' in that they initially ask questions which are not directly related to production issues, such as; longitudinal changes and interactions. However, in order to implement successful integrated management strategies involving optimal selection protocol it is important to investigate the underlying biology of the traits to be utilised. In these chapters, as results appeared, many more questions and possible theories/explanations evolved. This section will discuss these questions and how they relate to possible breeding strategies.

Chapter 6 involved a different approach to parasitic infection and aimed to look at the parasitic community that may exist within a host species from a more epidemiological viewpoint. It was important to investigate this approach, as assessing the impact of breeding for resistance in terms of disease control is an epidemiological rather than a genetic question. This arises from the fact that with selective breeding strategies for enhanced disease resistance, we are interested

in the effects at the herd level, that is in the population dynamics rather than the individual animal effects. The consequence of this is that it will then be possible to assess the effect of breeding on the prevalence and severity of disease within the entire herd or flock.

When integrating genetic and epidemiological approaches the question can be raised as to how sustainable host genetics will be in controlling disease, when compared to other control strategies. It is problems arising from the failure of vaccines and drugs which are now necessitating new strategies to be sought. It is thought that sustainability may be a more pressing issue in the control of viral and bacterial disease, as has been demonstrated by the rapid development of drug resistant strains. On present knowledge it appears that, wherever possible, combined control strategies should be implemented in order to enhance the sustainability of all the component strategies.

When considering the sustainability of parasite control strategies the question of parasite evolution must also be considered. Using present knowledge it is impossible to determine the actual effect of parasite evolution on the sustainability of breeding programs. However it is possible to discuss how different control methods, in this case anthelmintics and breeding, may affect the rate of parasite evolution. When anthelmintics are the predominant control measure, treatment will kill all parasites except those with drug resistance. This creates a drug-resistant subpopulation which can then proliferate without hindrance, as subsequent treatments will have little effect. Thus anthelmintic treatment creates a strong selection pressure which can then result in a rapid rate of evolution in the parasite. This phenomenon has been widely reported as anthelmintics are now failing to control such populations.

However when we consider breeding as a control strategy we create an entirely different situation. When breeding for resistance the host population remains heterogeneous and thus displays a range of immune responses, and although the majority of the parasites are still harboured by the animals with the most susceptible genotypes, these genotypes are not fixed. Thus, in relation to the anthelmintic situation, selection pressure is slight and parasite evolution is likely to be slower. When drawing conclusions on the effectiveness of these methods in controlling parasitic infection is also important to consider the timeframe of the effects; anthelmintics will act with immediate effect, such is the nature of drug intervention methods, whereas the effect of selective breeding will be additive over generations. This again highlights the need for integrative strategies which combine the speed of drug intervention with the predicted sustainability of selective breeding.

Another issue which arose at many points throughout this study was that of trait definitions. It became increasingly obvious that to define a 'disease trait' or a 'resistance trait' could be highly subjective. This is a question which has been frequently raised concerning many livestock diseases; a particularly good example is mastitis in dairy cattle. For many years somatic cell count has been measured as an indicator of infection levels, however how can we be sure that when we measure this count at different stages of lactation or even between lactations that it is indeed the same trait? Regarding parasitic infection, this same reasoning may apply to faecal egg count, historically the preferred trait. As was raised in Chapter 6, how effective a measure of infection is this and what exactly does it reflect? This study suggests that between-animal differences in FEC in fact reflects a more accurate picture of between-animal variation in *Cooperia* infection and thus its use as an indicator of *T. circumcincta* infection

may well be more limited. The importance of this is based on the fact that the heritability of resistance is very much a function of trait definition, which increases as trait definition improves. Thus it becomes very important that breeding strategies are implemented only when trait definitions are as robust as is possible.

An important question raised in Chapter 3 was that of the optimal age for selection. We have just discussed the importance of robust traits yet how can we be sure that these traits remain consistent over time? And is there an optimal age to select for different traits? This is particularly relevant to the immunological indicator traits such as IgA activity and eosinophil count which may change according to the type of response the animal has mounted and also the time taken for the acquired immune response to develop. The results in chapter 3 suggest that there are significant changes occurring in terms of the biological interpretation of these traits and as such it is important that the optimal age for selection is investigated if these traits are to be utilised in a breeding scheme. There was a distinct lack of literature assessing the longitudinal changes in genetic correlations relating to such traits and hence few comparisons could be made to other studies.

7.4 Future research

Each chapter of this thesis has raised interesting questions, the answers to which will involve further research. The first aspect of work to further these investigations must be more QTL studies. It is important to discover if the same QTL observed in this study are segregating in other flocks, particularly commercial flocks such as Texel and Suffolk flocks, which may be under selection for meat quality and production traits. It would also be interesting to

search for QTL for traits which were shown to be heritable in chapters 2 and 3 but which were not included in the QTL analyses in this thesis; these would be traits such as eosinophil count and parasite development traits.

To expand upon the results presented in Chapter 4, it would be interesting to complete the genome scan as a partial scan was carried out – only a small number of chromosomes were genotyped due to financial constraints. Given the quality of the results obtained completing the genome scan may yield yet more interesting results. Also now that several QTL associated with parasitic infection have been identified, the next logical step would be to sequence underlying genes of interest, for example IFNG and several MHC candidates.

From QTL and host-parasite interaction views it would be interesting to study these interactions over a longer time period, for example 12 months, this would increase the understanding of the immune response development and also add to the evidence to support the optimum age for selection question. This would enhance our knowledge by allowing the study of the genetic correlations over a longer time period and it would also allow one to investigate if the same QTL are expressed at different measurement times, thus providing greater information as to how the genetic control of host-parasite interactions changes with host age.

Considering speculation regarding the sustainability of breeding as a control strategy, a logical progression from the previous discussion in section 7.3 would be to model likely rates of parasite evolution. Initially, one would develop models to compare anthelmintic intervention with selective breeding. Then a further step would be to investigate the effects of different selection procedures, for example QTL. This would allow us to investigate the power of selection without prior infection versus indicator trait selection, a direct reflection

of the immune response. This would provide an interesting comparison on how different selection protocol in the host animal may cause different selection pressures on the parasite. Given that each method appears at present to have advantages as well as disadvantages, sustainability is an important issue which may become even more important as anthelmintic resistance increases.

From my own experience it has become apparent that, as animal scientists, we can lose sight of the responsibility we have to society and as society's needs change we must be adaptable to these changes. In recent years the demands of the consumer have changed and the general public are now much more aware of issues such as drug residues in produce due to routine use of compounds such as anthelmintics and pesticides and animal welfare. By changing in line with the needs of the consumer, animal science remains dynamic and this thesis illustrates how disease control strategies can and must change in order to meet those needs. Thus, this is the time to create and implement industry-wide integrated control strategies at the herd level for parasitic infection management.

References

Albers,G.A.A., Gray,G.D., Piper,L.R., Barker,J.S.F., Lejambre,L.F. & Barger,I.A. 1987. The genetics of resistance and resilience to *Haemonchus contortus* infection in young Merino sheep. *International Journal for Parasitology* **17:** 1355-1363.

Amarante A.F, Craig T M, Ramsey W S, El-Sayed N M, Desouki A Y & Bazer F W 1999. Comparison of naturally acquired parasite burdens among Florida Native, Rambouillet and crossbred ewes. *Veterinary Parasitology* **85:** 61-69.

Armour,J., Jarrett,W.F.H. & Jennings,F.W. 1966. Experimental *Ostertagia circumcincta* infections in sheep: development and pathogenesis of a single infection. *American Journal of Veterinary Research* **27:** 1267-1278.

Armour,J. & Coop,R.L. 1991. Gastrointestinal parasites. In: *Diseases of Sheep* (Ed. by W.B.Martin & I.D.Aitken) Blackwell Scientific Publications.

Bahirathan M, Miller,J.E., Barras S R & Kearney M T 1996. Susceptibility of Suffolk and Gulf Coast Native suckling lambs to naturally acquired strongylate nematode infections. *Veterinary Parasitology* **65:** 259-268.

Bairden,K. 1991. Ruminant Parasitic Gastroenteritis: Some Observations on Epidemiology and Control. University of Glasgow.

Beh,K.J. & Maddox,J.F. 1996. Prospects for development of genetic markers for resistance to gastrointestinal parsite infection in sheep. *International Journal for Parasitology* **26:** 879-897.

Beh,K.J., Hulme,D.J., Callaghan,M.J., Leish,Z., Lenane,I., Windon,R.G. & Maddox,J.F. 2002. A genome scan for quantitative trait loci affecting resistance to *Trichostrongylus colubriformis* in sheep. *Animal Genetics* **33:** 97-106.

Behm,C.A. & Ovington,K.S. 2000. The role of eosinophils in parasitic helminth infection: insights from genetically modified mice. *Parasitology Today* **16:** 202-209.

Bernstein,R.E. 1987. Nonenzymatically glycosylated proteins. *Advances in Clinical Chemistry* **26:** 1-78.

Bishop,S.C., Bairden,K., McKellar,Q.A., Park,M. & Stear,M.J. 1996. Genetic parameters for faecal egg count following mixed, natural,predominantly *Ostertagia circumcincta* infection and relationships with live weight in young lambs. *Animal Science* **63:** 423-428.

Bishop,S.C. & Stear,M.J. 1997. Modelling responses to selection for resistance to gastro-intestinal parasites in sheep. *Animal Science* **64:** 469-478.

Bishop,S.C. & Stear,M.J. 1999. Genetic and epidemiological relationships between productivity and disease resistance: gastro-intestinal parasite infection in growing lambs. *Animal Science* **69:** 515-524.

Bishop,S.C. & Stear,M.J. 2000. The use of a gamma-type function to assess the relationship between the number of adult *Teladorsagia circumcincta* and total egg output. *Parasitology* **121:** 435-440.

Bishop,S.C. Breeding for Disease Resistance: Uniting Genetics and Epidemiology. 2002.

Bishop,S.C. & Stear,M.J. 2003. Modeling of host genetics and resistance to infectious diseases: understanding and controlling nematode infections. *Veterinary Parasitology* **115:** 147-166.

Bisset,S.A., Vlassof,A., Morris,C.A., Southey,B.R., Baker,R.L. & Parker,A.G.H. 1992. Heritability of and genetic correlations among faecal egg counts and productivity traits in Romney sheep. *New Zealand Journal of Agricultural Research* **35:** 51-58.

Bisset,S.A. & Morris,C.A. 1996. Feasibility and implications of breeding sheep for rsilience to nematode challenge. *International Journal for Parasitology* **26:** 857-868.

Bouix,J., Krupinski,J., Rzepecki,R., Nowosad,B., Skrzyzala,I., Roborzynski,M., Fudalewicz-Niemczyk,W., Skalska,M., Malczewski,A. & Gruner,L. 1998. Genetic resistance to gastrointestinal nematode parasites in Polish long-wool sheep. *International Journal for Parasitology* **28:** 1797-1804.

Buitkamp, JF, Stear, MJ, Epplen JT. 1996. Class I and class II major histocompatability complex alleles are associated with faecal egg counts following natural, predominantly *Ostertagia circumcincta* infection. *Parasitological Research* **82**: 693 – 696.

Chan M.S. 1997. The global burden of intestinal nematode infections – fifty years on. *Parasitology Today* **13(11):** 438-443.

Charon KM, Moskwa B, Rutkowski R, Gruszczynska J, Swiderek W. 2002. Microsatellite polymorphism in DRB1 gene (MHC Class II) and its relation to nematode faecal egg count in Polish Heath Sheep. *Journal of Animal and Feed Sciences* **11**: 47 – 58.

Churchill G A & Doerge R W. 1994. Empirical threshold values for quantitative trait mapping. *Genetics* **138:** 963-971.

Coltman,D.W., Wilson,K., Pilkington,J.G., Stear,M.J. & Pemberton,J.M. 2001. A microsatellite polymorphism in the gamma interferon gene is associated with resistance to gastrointestinal nematodes in a naturally-parasitized population of Soay sheep. *Parasitology* **122:** 571-582.

Coop,R.L., Sykes,A.R. & Angus,K.W. 1982. The effect of three levels of *Ostertagia circumcincta* larvae on growth rate, food intake and body composition of growing lambs. *Journal of Animal Science* **98:** 247-255.

Coop,R.L., Graham,R.B., Jackson,F., Wright,S.E. & Angus,K.W. 1985. Effect of experimental *Ostertagia circumcincta* infection on the performance of grazing lambs. *Research in Veterinary Science* **38:** 282-287.

Coop,R.L. & Holmes,P.H. 1996. Nutrition and parasite interaction. *International Journal for Parasitology* **26:** 951-962.

Courtney,C.H., Parker,C.F., McCLure,K.E. & Herd,R.P. 1985. Resistance of exotic and domestic lambs to experimental infection with *Haemonchus contortus*. *International Journal for Parasitology* **15:** 101-109.

Crawford,A.M. & McEwen,J.C. Identification of Animals Resistant to Nematose Parasite Infection. [330201]. 1998. New Zealand.

Datta,F.U., Nolan,J.V., Rowe,J.B., Gray,G.D. & 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.

Davies G, Stear,M.J. & Bishop,S.C. 2005. Genetic relationships between indicator traits and nematode parasite infection levels in 6 month old lambs. *Animal Science* **80:** 143-150.

Davies G., Bishop S.C. & Giuffra E. 2006. D08.1.1 A list of infectious pathogens and livestock diseases. European Animal Disease Genomics Network of Excellence for Animal Health and Food Safety: Project no : FOOD-CT-2004-506416.

Dawkins,H.J.S., Windon,R.G. & Eagleson,G.K. 1989. Eosinophil responses in sheep selected for high and low responsiveness to Trichostrongylus colubriformis. *International Journal for Parasitology* **19:** 199-205.

Dekkers,J.C.M. & Hospital,F. 2002. The use of molecular genetics in the improvement of agricultural populations. *Nature Reviews Genetics* **3:** 22-32.

Diez-Tascon C, Macdonald,P.A., Dodds,K.G., McEwan,J.C. & Crawford,A.M. A screen of chromosome 1 for QTL affecting nematode resistance in an ovine outcross population. 13-37. 2002. Proceedings 7th World Congress on Genetics Applied to Livestock Production.

Diez-Tascón C., Keane O.M., Wilson T., Zadissa A., Hyndman D.L., Baird D.B., McEwan J.C. and Crawford A.M. 2005. Microarray analysis of selection lines from outbred populations to identify genes involved with nematode parasite resistance in sheep. *Physiological Genomics* **21**: 56-69.

Dobson R.J. & Barnes E.H. 1995. Interaction between *Ostertagia circumcincta* and *Haemonchus contortus* infection in young lambs. *International Journal for Parasitology*. **25(4)**: 495-501

Doligalska,M., Moskwa,B. & Stear,M.J. 1999. Relationships among peripheral eosinophilia, eosinophil peroxidase activity, interleukin-5 concentration and faecal nematode egg count during natural, mixed gastrointestinal nematode infection. *Veterinary Immunology and Immunopathology* **70:** 299-308.

Douch,P.G.C., Green,R.S., Morris,C.A., Bisset,S.A., Vlassof,A., Baker,R.L., Watson,T.G., Hurford,A.P. & Wheeler,M. 1995. Genetic and phenotypic relationships among anti-*Trichostrongylus colubriformis* antibody level, faecal egg count and body weight traits in grazing Romney sheep. *Livestock Production Science* **41:** 121-132.

Douch,P.G.C., Green,R.S., Morris,C.A., McEwan,J.C. & Windon,R.G. 1996. Phenotypic markers for selection of nematode-resistant sheep. *International Journal for Parasitology* **26:** 899-911.

Eady,S.J. Worm resistance in a merino breeding objective - influence of genetic correlations between resistance and production traits. 27: 141-144. 1998. Proceedings of the 6th World Congress On Genetics and Applied Livestock Production.

Else,K.J., Wakelin,D., Wassom,D.L. & Hauda,K.M. 1990. The influence of genes mapping within the major histocompatibility complex on resistance to *Trichuris muris* infections in mice. *Parasitology* **101:** 61-67.

Fox,M.T., Gerrelli,D., Shivalkar,P. & Jacobs,D.E. 1989. Effect of omeprazole treatment on feed intake and blood gastrin and pepsinogen levels in the calf. *Research in Veterinary Science* **46:** 280-282.

Gamble,H.R. & Zajac,A.M. 1992. Resistance of St Croix lambs to *Haemonchus contortus* in experimentally and naturally acquired infections. *Veterinary Parasitology* **41:** 211-225.

Gauly,M., Kraus,M., Vervelde,L., van Leeuwen,M.A.W. & Erhardt,G. 2002. Estimating genetic differences in natural resistance in Rhön and Merinoland sheep following experimental Haemonchus contortus infection. *Veterinary Parasitology* **106:** 55-67.

Geczy,A.F. & Rothwell,T.L. 1981. Genes within the major histocompatibility complex of the guinea pig influence susceptibility to *Trichostrongylus colubriformis* infection. *Parasitology* **82:** 281-286.

GENESHEEPSAFETY. 2006. Final Report on EU Project GENESHEEPSAFETY (QLK5-CT-2000-00656). Barillet F., Bishop S.C., Arranz J.J., Stear M.J., Jacquiet P. & Carta A.

Gill,H.S. 1991. Genetic control of acquired resistance to haemonchosis in Merino lambs. *Parasite Immunology* **13:** 617-628.

Gilmour,A.R., Thompson,R., Cullis,B.R. & Welham,S. ASREML. 1996. Biometrics Bulletin 3, NSW Agriculture.

Gordon,H.M. & Whitlock,H.V. 1939. A new technique for counting nematode eggs in sheep faeces. *Journal of the Council for Scientific and Industrial Research, Australia* **12:** 50.

Gray,G.D. 1987. Genetic resistance to haemonchosis in sheep. *Parasitology Today* **3:** 253-255.

Green P, Falls K & Crooks S . Cri-map Version 2.4. [2.4]. 1990. St. Louis, MO., Washington University School of Medicine.

Gruner,L., Bouix,J., Cabaret,J., Boulard,C., Cortet,J., Sauve,C., Molenat,G. & Calamel,M. 1992. Effect of genetic type, lactation and management on helminth infection of ewes in an intensive grazing system on irrigated pasture. *International Journal for Parasitology* **22:** 919-925.

Heath,M.F. & Connan,R.M. 1991. Interaction of ostertagia and nematodirus species in sheep and the potential of serum fructosamine determination in monitoring gastrointestinal parasitism. *Research in Veterinary Science* **51:** 322-326.

Hulme,D.J., Nicolas,F.W., Windon,R.G., Brown,S.C. & Beh,K.J. 1993. The MHC class II region and resistance to an intestinal parasite in sheep. *Journal of Animal Breeding and Genetics* **110:** 459-472.

Hutchings,M.R., Kyriazakis,I., Anderson,D.H., Gordon,I.J. & Coop,R.L. 1998. Behavioural strategies used by parasitized and non-parasitized sheep to avoid ingestion of gastro-intestinal nematodes associated with faeces. *Animal Science* **67:** 97-106.

Hutchings,M.R., Kyriazakis,I., Gordon,I.J. & Jackson,F. 1999. Trade-offs between nutrient intake and faecal avoidance in herbivore grazing decisions: the effect of animal parasitic status, level of feeding motivation and sward nitrogen content. *Animal Ecology* **68:** 310-323.

Iraqi F.A., Behnke J.M., Menge D.M., Lowe A., Teale A.J., Gibson J.P., Baker L.R. & Wakelin D. 2003. Chromosomal regions controlling resistance to gastro-intestinal nematode infections in mice. *Mammalian Genome* **14**: 184-191.

Jackson F. & Coop R.L. 2000. The development of anthelmintic resistance in sheep nematodes. *Parasitology* **120**: S95-S107.

Janeway,C.A., Travers,P., Walport,M. & Schlomchik,M. 2001. *Immunobiology 5.* New York: Garland Publishing.

Janssen M, Weimann C, Gauly,M. & Erhardt,G. Associations between infections with Haemonchus contortus and genetic markers on ovine chromosome 20. 13-11. 2002. Proceedings 7th World Congress on Genetics Applied to Livestock Production.

Kennedy,M.W. 1989. Genetic control of the immune repertoire in nematode infections. *Parasitology Today* **5:** 316-324.

Kimambo,A.E., Macrae,J.C. & Dewey,P.J.S. 1998. The effect of daily challenge with *Trichostrongylus colubriformis* larvae on the nutrition and performance of immuologically-resistant sheep. *Veterinary Parasitology* **28:** 205-212.

Knott S A, Elsen J-M & Haley,C.S. 1996. Methods for multiple-marker mapping of quantitative trait loci in half-sib populations. *Theoretical Applied Genetics* **93:** 71-80.

Knott S A, Marklund L, Haley,C.S., Andersson K, Davies W, Ellegren H, Fredholm M, Hansson I, Hoyheim B, Lundstrom K, Moller M & Andersson L 1998. Multiple Marker Mapping of Quantitative Trait Loci in a Cross Between Outbred Wild Boar and Large White Pigs. *Genetics* **149:** 1069-1080.

Kooyman F.N.J., Schallig H.D.F.H., Van Leeuwen M.A.W., Mackellar A., Huntley J.F., Cornelissen A.W.C.A., Vervelde L. 2000. Protection in lambs vaccinated

with Haemonchus contortus antigens is age related, and correlates with IgE rather than IgG1 antibody. *Parasite Immunology*, **22(1)**: 13-20.

Lello J., Boag B., Fenton A., Stevenson I.R. & Hudson P.J. 2004. Competition and mutualism among the gut helminthes of a mammalian host. *Nature* **428**: 840-844

Li Y, Miller,J.E. & Franke D E 2001. Epidemiological observation and heterosis of gastrointestinal nematode infection in Suffolk, Gulf Coast Native and crossbred lambs. *Veterinary Parasitology* **98:** 273-283.

Luffau,G., Vu Tien Khang,J., Bouix,J., Nguyen,T.G., Cullen,P. & Ricordeau,G. 1990. Reistance to experimental infections with Haemonchus contortus in Romanov sheep. *Genetics,Selection and Evolution* **22:** 205-229.

Lunney,J.K. & Murrell,K.D. 1988. Immunogenetic analysis of *Trichinella spiralis* infections in swine. *Veterinary Parasitology* **29:** 170-193.

Lynch M. & Walsh B. 1998. *Genetics and analysis of quantitative traits*. Sinauer Associates.

Macpherson A.J., Gatto D., Sainsbury E., Harriman G.R., Hengartner H., & Zinkernagel R.M. 2000. A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. *Science* **288(5474):** 2222-2226.

Maillard, J.C., Chantal, I., Berthier, D., Sidibé, I., Stachurski, F. Razafindraïbé, H. and Elsen, J.M. 2002. Eugenic selection assisted by a BoLA-DR/DQ haplotype associated with susceptibility to bovine dermatophilosis. http://cgah.cirad.fr/pdf/maillard_5.pdf

Maillard, J.C., Chantal, I., Berthier, D., Stachursky, F. and Elsen, J.M. 1999. Molecular markers of genetic resistance and susceptibility to bovine dermatophilosis. *Archive Animal Breeding*. **42**: S93-S96.

McCririe,L., Bairden,K., Britton,C., Buitkamp,J., McKeand,J.B. & Stear,M.J. 1997. Heterogeneity in the recognition of *Ostertagia circumcincta* antigens by serum antibody from mature, infected sheep. *Parasite Immunology* **19:** 235-242.

McEwan,J.C., Mason,P., Baker,R.L., Clarke,J.N., Hickey,S.M. & Turner,K. 1992. Effect of selection for productive traits on internal parasite resistance in sheep. *Proceedings of the New Zealand Society of Animal Production* **52:** 53-56.

McEwan,J.C., Dodds,K.G., Greer,G.J., Bain,W.E., Duncan,S.J., Wheeler,R., Knowler,K.J., Reid,P.J., Green,R.S. & Douch,P.G.C. 1995. Genetic estimates for parasite resistance traits in sheep and their correlations with production traits. *New Zealand Journal of Zoology* **22:** 177.

McKellar,Q.A., Duncan,J.L., Armour,J. & McWilliam,P. 1986. Response to transplanted adult *Ostertagia ostertagi* in calves. *Research in Veterinary Science* **40:** 367-371.

Mcleod R.S. 1995. Costs of major parasites to the Australian livestock industries. *International Journal for Parasitology* **25(11):** 1363-1367.

Meeusen,E.N.T. & Balic,A. 2000. Do eosinophils have a role in the killing of helminth parasites? *Parasitology Today* **16:** 95-101.

Merck Veterinary Manual 9th Ed. 2005. Ed. Line S. Merial.

Meuwissen T.H.E. & Van Arendonk J.A.M. 1992. Potential Improvements in Rate of Genetic Gain from Marker-Assisted Selection in Dairy Cattle Breeding Schemes. *Journal of Dairy Science* **75(6):**1651-1659.

Miller,J.E., Bahirathan M, Lemarie S L, Hembry F G, Kearney M T & Barras S R 1998. Epidemiology of gastrointestinal nematode parasitism in Suffolk and Gulf Coast Native sheep with special emphasis on relative susceptibility to *Haemonchus contortus* infection. *Veterinary Parasitology* **74:** 55-74.

Moreno C.R., Gruner L., Scala A., Mura L., Schibler L., Amigues Y., Sechi T., Jacquiet P., Francois D., Sechi S., Roig A., Casu S., Barillet F., Brunel J.C., Bouix J., Carta A. & Rupp R. 2006. QTL for resistance to internal parsites in tow designs based on natural and experimental conditions of infection. *Proceedings 8th World Congress on Genetics Applied to Livestock Production.*

Morris,C.A., Vlassof,A., Bisset,S.A., Baker,R.L. & Watson,T.G. 1997. Direct responses to selection for divergence in faecal nematode egg count in young Romney and Perendale sheep. *Proceedings of the Association for the advancement of Animal Breeding and Genetics* **12:** 413-416.

Morris,C.A., Vlassof,A., Bisset,S.A., Baker,R.L., West,C.J. & Hurford,A.P. 1997. Responses of Romney sheep to selection for resistance or susceptibility to nematode infection. *Animal Science* **64:** 319-329.

Morris,C.A., Green,R.S., Cullen,N.G. & Hickey,S.M. 2003. Genetic and phenotypic relationships among faecal egg count, anti-nematode antibody level and live weight in Angus cattle. *Animal Science* **76:** 167-174.

Munn,E.A. 1993. Development of a vaccine against *Haemonchus contortus*. *Parasitology Today* **9:** 338-339.

Murray,D.L., Cary,J.R. & Keith,L.B. 1997. Interactive effects of sublethal nematodes and nutritional status on snowshoe hare vulnerability to predation. *Journal of Animal Ecology* **66:** 250-264.

Nicolas,F.W. 1987. *Veterinary Genetics*. Oxford University Press.

Noble.E.R, Noble,G.A., Schad,G.A. & Macinnes,A.J. 1989. *Parasitology the Biology of Animal Parasites*. Lea & Febiger.

Nieuwhof G.J. and Bishop S.C. 2005. Costs of the major endemic diseases of sheep in Great Britain and the potential benefits of reduction in disease impact. *Animal Science* **81(1):** 23-29.

Outteridge,P.M., Windon,R.G. & Dineen,J.K. 1985. An association between a lymphocyte antigen in sheep and the response to vaccination against the parasite Trichostrongylus colubriformis. International Journal for Parasitology **15:** 121-127.

Outteridge,P.M., Windon,R.G., Dineen,J.K. & Smith,E.F. 1986. The relationship between ovine lymphocyte antigens and faecal egg count of sheep selected for responsiveness to vaccination against *Trichostrongylus colubriformis. International Journal for Parasitology* **16:** 369-374.

Outteridge,P.M., Windon,R.G. & Dineen,J.K. 1988. An ovine lymphocyte antigen marker for acquired resistance to *Trichostrongylus colubriformis. International Journal for Parasitology* **18:** 853-858.

Paterson S, Wilson K, Pemberton JM. 1998. Major histocompatability complex variation associated with juvenile survival ad parasite resistance in a large

ungulate population (*Ovis aries* L.). *Proceedings of the National Academy of Sciences* **95(7)**: 3714 – 3719.

Paterson,K.A., McEwan,J.C., Dodds,K.G., Morris,C.A. & Crawford,A.M. 1999. Fine mapping a locus affecting host resistance to internal parasites in sheep. Proceedings of the Association for Advancement in Animal Breeding and Genetics. **13:** 91-94.

Paterson,K.A., McEwan,J.C., Dodds,K.G., Morris,C.A. & Crawford,A.M. 2001. Fine mapping a locus affecting host resistance to internal parasites in sheep, in: *Proceedings of the Association for Advancement in Animal Breeding and Genetics,* 30 July – 2 August. Vol. **13**, Queesnstown, New Zealand. 91-94

Paynter,D.I. 1992. Australian Standard Diagnostic Techniques for Animal Diseases. Standing Committee of Agriculture. Australia, CSIRO.

Petney T.N. & Andrews R.H. 1998. Multiparasite communities in animals and humans: frequency, structure and pathogenic significance. *International Journal of Parasitology.* **28**: 377-393.

Preston,J.M. & Allonby,E.W. 1979. The influence of breed on the susceptibility of sheep to *Haemonchus contortus* infection in Kenya. *Research in Veterinary Science* **26:** 134-139.

Quinnell R J. 2003. Genetics of susceptibility to human helminth infection. *International Journal for Parasitology* **33:** 1219-1231.

Radhakrishnan C V, Bradley R E & Loggins P E. 1972. Host responses of worm-free Florida Native and Rambouillet lambs experimentally infected with *Haemonchus contortus. American Journal of Veterinary Research* **33:** 817-823.

Rothwell,T.L.W., Windon,R.G., Horsburgh,B.A. & Anderson,B.H. 1993. Relationship between eosinophilia and responsiveness to infection with *Trichostrongylus colubriformis* in sheep. *International Journal for Parasitology* **23:** 203-211.

Schwaiger,F.W., Gostomski,D., Stear,M.J., Duncan,J.L., McKellar,Q.A., Epplen,J.T. & Buitkamp,J. 1995. An ovine *Major Histocompatibilty Complex* DRB1 allele is associated with low faecal egg counts following natural, predominantly *Ostertagia circumcincta* infection. *International Journal for Parasitology* **25:** 815-822.

Seaton G, Haley,C.S., Knott S A, Kearsey M & Visscher P M 2002. QTL Express: mapping quantitative trait loci in simple and complex pedigrees. *Bioinformatics* **18:** 339-340.

Seaton,D.S. 1989. Development of immunity to incoming radiolabelled larvae in lambs continuously infected with *Ostertagia circumcincta*. *Research in Veterinary Science* **46:** 241-246.

Shaw,R.J., Morris,C.A., Green,R.S., Wheeler,M., Bisset,S.A., Vlassof,A. & Douch,P.G.C. 1999. Genetic and phenotypic relationships among *Trichostrongylus colubriformis* - specific immunoglobulin E, anti - *Trichostrongylus colubriformis* antibody, immunoglubulin G_1, faecal egg count and body weight traits in grazing Romney lambs. *Livestock Production Science* **58:** 25-32.

Shay,T.L., Miller,J.E., McGraw,R.A., Walling,G.A., Bishop,S.C., Haley,C.S. & Cockett,N.E. Characterization of QTL associated with resistance to nematode

infection in sheep. 189. 2002. XXVIII International Conference on Animal Genetics. 11-8-2002.

Sinski,E., Bairden,K., Duncan,J.L., Eisler,M.C., Holmes,P.H., McKellar,Q.A., Murray,M. & Stear,M.J. 1995. Local and plasma antibody-responses to the parasitic larval stages of the abomasal nematode Ostertagia circumcincta. *Veterinary Parasitology* **59:** 107-118.

Smith,N.C. 1992. Concepts and strategies for antiparasite immunoprophylaxis and therapy. *International Journal for Parasitology* **22:** 1047-1082.

Smith,W.D., Jackson,F., Jackson,E. & Williams,J. 1985. Age immunity to *Ostertagia circumcincta*: comparison of the local immune responses of 4.5 and 10 month-old lambs. *Journal of Comparative Pathology* **95:** 235-245.

Smith,W.D. 1999. Prospects for vaccines of helminth parasites of grazing ruminants. *International Journal for Parasitology* **29:** 17-24.

Stear,M.J., Tierney,T.J., Baldock,F.C., Brown,S.C., Nicolas,F.W. & Rudder,T.H. 1988. Class I antigens of the bovine major histocompatibilty system are weakly associated with variation in faecal worm egg counts in naturally infected cattle. *Animal Genetics* **19:** 115-122.

Stear,M.J., Baldock,F.C., Brown,S.C., Gershwin,L.J., Hetzel,D.J.S., Miller,J.E., Nicolas,F.W., Rudder,T.H. & Tierney,T.J. 1990a. The genetic control of nematode infections in ruminants. *Fourth World Congress on Genetics Applied to Livestock Production, Edinburgh* **16:** 449-452.

Stear,M.J., Hetzel,D.J.S., Brown,S.C., Gershwin,L.J., Mackinnon,M.J. & Nicolas,F.W. 1990b. The relationships among ecto- and endoparasite levels,

class I antigens of the bovine major histocompatibility system, immunoglobulin E levels and weight gain. *Veterinary Parasitology* **34:** 303-321.

Stear,M.J. & Murray,M. 1994. Genetic resistance to parasitic disease: particularly of resistance in ruminants to gastrointestinal. *Veterinary Parasitology* **54:** 161-176.

Stear,M.J., Bairden,K., Duncan,J.L., Gettinby,G., McKellar,Q.A., Murray,M. & Wallace,D.S. 1995a. The distribution of faecal nematode egg counts in Scottish Blackface lambs following natural, predominantly *Ostertagia circumcincta* infection. *Parasitology* **110:** 573-581.

Stear,M.J., Bishop,S.C., Doligalska,M., Duncan,J.L., Holmes,P.H., Irvine,J., McCririe,L., McKellar,Q.A., Sinski,E. & Murray,M. 1995b. Regulation of egg production, worm burden, worm length and worm fecundity by host responses in sheep infected with *Ostertagia circumcincta*. *Parasite Immunology* **17:** 643-652.

Stear,M.J., Bishop,S.C., Duncan,J.L., McKellar,Q.A. & Murray,M. 1995c. The Repeatability of Faecal Egg Counts, Peripheral Eosinophil Counts and Plasma Pepsinogen Concentrations During Deliberate Infections with *Ostertagia circumcincta*. *International Journal for Parasitology* **25:** 375-380.

Stear,M.J., Park,M. & Bishop,S.C. 1996. The key components of resistance to Ostertagia circumcincta in lambs. *Parasitology Today* **12:** 438-441.

Stear,M.J., Bairden,K., Duncan,J.L., Holmes,P.H., McKellar,Q.A., Park,M., Strain,S., Murray,M., Bishop,S.C. & Gettinby,G. 1997a. How hosts control worms. *Nature* **389:** 27.

Stear,M.J., Bairden,K., Bishop,S.C., Buitkamp,J., Duncan,J.L., Gettinby,G., McKellar,Q.A., Park,M., Parkins,J.J., Reid,S.W.J., Strain,S. & Murray,M. 1997b. The Genetic Basis of Resistance to *Ostertagia circumcincta* in Lambs. *The Veterinary Journal* **154:** 111-119.

Stear,M.J., Bairden,K., Bishop,S.C., Gettinby,G., McKellar,Q.A., Park,M., Strain,S. & Wallace,D.S. 1998. The processes influencing the distribution of parasitic nematodes among naturally infected lambs. *Parasitology* **117:** 165-171.

Stear,M.J., Strain,S. & Bishop,S.C. 1999. Mechanisms underlying resistance to nematode infection. *International Journal for Parasitology* **29:** 51-56.

Stear,M.J. & Bishop,S.C. 1999. The curvilinear relationship between worm length and fecundity of *Teladorsagia circumcincta. International Journal for Parasitology* **29:** 777-780.

Stear,M.J., Bairden,K., McKellar,Q.A., Scott,I., Strain,S. & Bishop,S.C. 1999. The relationship between the number and size of nematodes in the abomasum and the concentration of pepsinogen in ovine plasma. *Research in Veterinary Science* **67:** 89-92.

Stear,M.J., Eckersall,P.D., Graham,P.A., McKellar,Q.A., Mitchell,S. & Bishop,S.C. 2001a. Fructosamine concentration and resistance to infection with *Teladorsagia circumcincta* . *Parasitology* **123:** 211-218.

Stear,M.J., Bishop,S.C., Mallard,B.A. & Raadsma,H. 2001b. The sustainability, feasibility and desirability of breeding livestock for disease resistance. *Research in Veterinary Science* **71:** 1-7.

Stear,M.J., McKellar,Q.A., Mitchell,S., Seeley,C. & Bishop,S.C. 2002. Eosinophilia as a marker of resistance to *Teladorsagia circumcincta* in Scottish Blackface lambs. *Parasitology* **124:** 553-560.

Stear,M.J., Bairden,K., Innocent G.T., Mitchell S., Strain,S. & Bishop,S.C. 2004. The relationship between IgA activity against 4th-stage larvae and density-dependent effects on the number of 4th-stage larvae of *Teladorsagia cirumcincta* in naturally infected sheep. *Parasitology* **129**: 363-369.

Stear,M.J., Abuagob,O., Benothman,M., Bishop,S.C., Innocent,G., Kerr,A. & Mitchell,S. 2005. Variation among faecal egg counts following natural nematode infection in Scottish Blackface lambs. *Parasitology* **132(2):** 275-280.

Stevenson,L.M., Huntley,J.F., Smith,W.D. & Jones,D.G. 1994. Local eosinophil- and mast cell-related responses in abomasal nematode infections of lambs. *FEMS Immunology and Medical Microbiology* **8:** 167-174.

Strain,S., Bishop,S.C., Henderson,N.G., Holmes,P.H., McKellar,Q.A., Mitchell,S. & Stear,M.J. 2002. The genetic control of IgA activity and its association with parasite resistance in naturally infected sheep. *Parasitology* **124:** 545-552.

Sykes A.R. 1994. Parasitism and production in farm animals. *Animal Production* **59**: 155-172.

Tizard I R. 2004. *Veterinary Immunology An Introduction* 7th Ed. Saunders, Pennsylvania.

Urban J F, Fayer R, Sullivan C, Goldhill J, Shea-Donohue T, Madden K, Morris S C, Katona I, Gause W, Ruff M, Mansfield L S & Finkelman F D 1996. Local

TH1 and TH2 responses to parasitic infection in the intestine: regulation by IFN-gamma and IL-4. *Veterinary Immunology and Immunopathology* **54:** 337-344.

Urquhart,G.M., Armour,J., Duncan,J.L., Dunn,A.M. & Jennings,F.W. 1991. *Veterinary Parasitology*. Blackwell Publishing Professional.

van der Waaij,E.H., Bijma,P., Bishop,S.C. & van Arendonk,J.A.M. 2000. Modeling selection for production traits under constant infection pressure. *Journal of Animal Science* **78:** 2809-2820.

van der Waaij,E.H., Bijma,P., Bishop,S.C. & van Arendonk,J.A.M. 2002. Using genetic markers for disease resistance to improve production under constant infection pressure. *Journal of Animal Science* **80:** 322-329.

van Egmond M, Damen CA, van Spriel AB, Vidarsson G, van Garderen E, van de Winkel JG. 2001. IgA and the IgA Fc receptor. *Trends in Immunology* **22(10)**:545-546.

Visscher P M, Thompson,R. & Haley,C.S. 1996. Confidence intervals in QTL mapping by bootstrapping. *Genetics* **143:** 1013-1020.

Wakelin,D. 1996. *Immunity to Parasites; How Parasitic Infections Are Controlled.* Cambridge: Cambridge University Press.

Wakelin,D.M. & Blackwell,J.M. 1988. *Genetics of Resistance to Bacterial and Parasitic Infection*. London: Taylor & Francis.

Waller,P.J. 1994. The development of anthelmintic resistance in ruminant livestock. *Acta Tropica* **56:** 233-243.

Waller P.J. 1997. Anthelmintic resistance. *Veterinary Parasitology* **72**: 391-412.

Walsh,G.M. 1999. Advances in the immunobiology of eosinophils and their role in disease. *Critical Reviews in Clinical Laboratory Sciences* **36:** 453-496.

Whitlock,H.V. 1948. Some modifications of the McMaster helminth egg-counting technique apparatus. *J. Council Sci. Ind. Res.* **21:** 177-180.

Whittier,W.D., Zajac,A.M. & Umberger,S.H. Control of Internal Parasites in Sheep. 410-027: 1-8. 1995. Virginia Cooperative Extension, Virginia Polytechnic Institute and State University.

Wiltshire,S., Bhattacharyya,S., Faux,J.A., Leaves,N.I., Daniels,S.E., Moffatt,M.F., James,A., Musk,A.W. & Cookson,W.O.C.M. 1998. A genome scan for loci influencing total serum immunoglobulin levels: possible linkage of IgA to the chromosome 13 atopy locus. *Human Molecular Genetics* **7:** 27-31.

Woolaston,R.R. & Piper,L.R. 1996. Selection of Merino sheep for resistance to *Haemonchus contortus* : genetic variation. *Animal Science* **62:** 451-460.

Woolaston,R.R. & Baker,R.L. 1996. Prospects of breeding small ruminants for resistance to internal parasites. *International Journal for Parasitology* **26:** 845-855.

Woolaston,R.R., Manueli,P., Eady,S.J., Barger,I.A., Le Jambre,L.F., Banks,D.J.D. & Windon,R.G. 1996. The value of circulating eosinophil count as a selection criterion for resistance of sheep to trichostrongyle parasites. *International Journal for Parasitology* **26:** 123-126.

Woolaston,R.R. & Windon,R.G. 2001. Selection of sheep for response to *Trichostrongylus colubriformis* larvae: genetic parameters. Animal Science **73**: 41-48.

Zinkernagel,R.M. & Doherty,P.C. 1979. MHC-restricted T cells: studies on the biological role of the polymorphic major transplantation antigens determining T-cell restriction-specificity, function and responsiveness. *Advances in Immunology* **27:** 221-291.