Caprine Responsiveness towards Gastrointestinal Nematode Infection.

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Abstract

Studies were conducted using Scottish cashmere goats which were segregated into responsive and non-responsive individuals on the basis of ranked faecal egg counts following artificial and natural gastrointestinal nematode infection. These studies demonstrate that caprine responsiveness to gastrointestinal nematode infection is a relatively stable and heritable characteristic, largely unaffected by season and mode or site of infection.

Initial comparative studies showed does to be considerably more susceptible to mixed artificial Teladorsagia circumcincta and Trichostrongylus vitrinus infection than were ewes or worm-naïve lambs. This was reflected in distinct differences in mucosal mast cell (MMC) and globule leukocyte (GL) populations, the does having more GLs but many fewer MMCs than ewes. These differences together with the very low sheep mast cell proteinase concentrations recovered from doe tissue suggest that there are important functional differences in the mast cell responses of sheep and goats.

The responses of breeding male and female goats were very consistent, with individuals occupying the same position of relative responsiveness while on pasture and after artificial challenge. Differences in the susceptibility of responders and non-responders were apparent in egg count following natural and artificial infection and selection was largely supported by worm burdens recovered after artificial challenge. There was a tendency for enhanced responsiveness to be associated with increased tissue eosinophil and GL numbers though this relationship was not very strong. However, responders were able to mount a more rapid and vigorous peripheral
eosinophil response than were non-responders suggesting that peripheral eosinophil levels may be indicative of the ability of the host to respond to infection. Analysis of the cellular traffic of the gastric lymph showed that more resistant individuals were responding earlier.

Results obtained from the first generation yearlings from the helminth-line showed that under the conditions encountered in these studies increased resistance to gastrointestinal nematode infection in Scottish cashmere goats is a heritable phenomenon with a heritability estimate (0.37) similar to those of production traits for which selection has been successful. Over the early stages of the programme selection for enhanced resistance appears to have had no detrimental effect upon productivity.
Declaration

The work described in this thesis was conducted at the Parasitology Division of the Moredun Research Institute, Edinburgh. The lymphatic cannulation surgery described in Chapter 5 was performed by Drs. E. W. Scott and F. Jackson of the Clinical and Parasitology departments, respectively. Some of the eosinophil and post-mortem tissue data was obtained in collaboration with colleagues at the Institute. Nevertheless, most of the work presented in this thesis was conducted by myself, and where conjoint experiments were undertaken, a full role was played in the design of the experiments and interpretation of the results.

David Mark Patterson
Moredun Research Institute
July, 1996
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Over the course of these studies there are a number of people who have been involved directly or indirectly and without whom this would not have been possible, or as much fun!

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<td>anthelmintic disrupted challenge model</td>
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<tr>
<td>BoLA</td>
<td>bovine lymphocyte antigen</td>
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<td>CCM</td>
<td>continuous challenge model</td>
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<tr>
<td>EDTA</td>
<td>Ethylenediaminetetracetate</td>
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<tr>
<td>epg</td>
<td>eggs per gramme of faeces</td>
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<tr>
<td>ECF-A</td>
<td>eosinophil chemotactic factor of anaphylaxis</td>
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<td>EL₄</td>
<td>early fourth-stage larva</td>
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<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<td>EPA</td>
<td>eosinophil potentiating activity</td>
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<td>ESCA</td>
<td>East of Scotland College of Agriculture</td>
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<td>FEC</td>
<td>faecal egg count</td>
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<td>FECRT</td>
<td>faecal egg count reduction test</td>
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<td>FHL</td>
<td>females helminth-line</td>
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<td>FUFL</td>
<td>females unselected fibre-line</td>
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<td>g</td>
<td>gravity</td>
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<td>GL</td>
<td>globule leukocyte</td>
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<td>L₁</td>
<td>first-stage larva</td>
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<td>L₂</td>
<td>second-stage larva</td>
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<td>L₃</td>
<td>third-stage larva</td>
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<td>LT</td>
<td>leukotriene</td>
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<td>mid fourth-stage larva</td>
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<td>MMC</td>
<td>mucosal mast cell</td>
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<td>MHC</td>
<td>major histocompatibility complex</td>
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<td>MUFL</td>
<td>males unselected fibre-line</td>
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<td>OD</td>
<td>optical density</td>
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<td>OLA</td>
<td>ovine lymphocyte antigen</td>
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<td>orthophenylenediamine</td>
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<td>PBS</td>
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<td>PI</td>
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<td>RFLP</td>
<td>restriction fragment length polymorphisms</td>
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<td>SEM</td>
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<td>sheep mast cell proteinase</td>
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<td>SRS-A</td>
<td>slow reacting substance of anaphylaxis</td>
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<td>UFL</td>
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<td>VCU</td>
<td>villus crypt unit</td>
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Chapter 1 - Introduction
1.1 Introduction

In terms of numbers of individuals, members of the phylum Nematoda are among the most abundant animals known. Though the vast majority of nematodes are free-living marine, freshwater or soil-dwelling species, many are important parasites of plants and animals. Of the latter, the species comprising the family Trichostrongylidae, which infect the ruminant gastrointestinal tract, are of the greatest veterinary importance.

The results of the last available survey indicate that there were just over 600 million goats world-wide compared to almost 1100 million sheep and 1300 million cattle (FAO/OIE/WHO, 1994). The majority of these goats (approximately 97%) are in Third World countries, where they are kept in small numbers to support the local community and as such have little direct commercial importance. In developed countries goats account for only 5% of small ruminants (FAO/OIE/WHO, 1994). The most recent survey conducted by the Meat and Livestock Commission (MLC) for the UK estimates that in 1995 this figure was much less than 1%, with approximately 84,000 goats kept on smallholdings in the UK. Of these, 10,500 were in Scotland (MLC, personal communication). The major Scottish goat farms are shown in Figure 1.1. Nevertheless, in recent years goats have become increasingly more attractive economically as an alternative to hill sheep, and herds are now kept for both fibre and meat. Currently, goat farming in the UK does not qualify for the EU subsidies available for sheep farming. However, any future changes involving either a reduction in the subsidy paid to sheep farmers or the introduction of a subsidy for goat farming are likely to greatly improve the economic viability of goat farming.
Figure 1.1. Location of sheep and goat farms in Scotland.
Gastrointestinal nematode infection in ruminants is commonly associated with high stocking densities and intensive animal husbandry systems. Trichostrongylid infection in particular is responsible for significant economic losses in ruminant production world-wide (Holmes, 1985). Gastrointestinal nematode infection has a detrimental effect upon the quantity and/or quality of wool (Barger and Southcott, 1975; Lipson and Bacon-Hall, 1976) and milk (Kloosterman, Borgsteede and Eysker, 1985; Hoste and Chartier, 1993) yield. Additional production losses may be sustained through lower liveweight gains, reduced fertility and birth weights and in extreme cases animal mortality (Barger, 1982). To these direct costs must be added the significant indirect costs of drug treatment and non-profitable pasture spelling in an attempt to reduce infection.

The economic consequences of parasite infection can be considerable. It has been estimated that in 1985 Ostertagia ostertagi infection alone cost the American cattle industry $600 million (Smith and Granfell, 1985). A similar study conducted in 1985 suggested that in an average year, parasitism cost the Australian sheep industry A$309 million in reduced production and A$53 million in chemotherapeutic control (Beck, Moir and Meppem, 1985). This equated to a loss of almost A$7500 per sheep farm. Internal parasitism was the most important source of loss accounting for two-thirds of the total. Of the costs due to internal parasitism over half were as a consequence of reduced wool production (Beck et al., 1985). To put this figure into context, total losses due to helminth infection were estimated to be approximately A$450 million in a year where the total value of wool exports was A$2400 million (Gray, 1987). Annual losses due to internal parasitism in sheep in New Zealand have
been estimated to be NZ$270 million with a further NZ$40 million lost due to the prevalence of fleece dags (New Zealand Wool Board, 1990).

1.1.2 Prevalence, life-cycle and epidemiology

Though there are a number of species of nematodes which parasitise the ruminant gastrointestinal tract the economic impact of many of these are limited by their climatic and host range. In temperate areas such as northern Britain the most prevalent gastrointestinal nematodes of small ruminants are Teladorsagia (Ostertagia) circumcincta, Trichostrongylus vitrinus and Nematodirus battus (Parnell, Rayski, Dunn and MacKintosh, 1954; Boag and Thomas, 1971, 1977; Thomas and Boag, 1972, 1973; Waller and Thomas, 1981). Of these the most economically significant to the UK sheep industry is the abomasal parasite T. circumcincta (Urquhart, Armour, Duncan, Dunn and Jennings, 1987), though T. vitrinus may be one of the main causes of helminthiasis in grazing lambs in their first winter (Parnell et al., 1954). Others such as Trichostrongylus colubriformis, Trichostrongylus axei, Haemonchus contortus, Chabertia ovina, Oesophagostomum venulosum, Nematodirus filicollis, Nematodirus spathiger, Trichuris ovis, Bunostomum trigonocephalum and Strongyloides papillosus tend to occur less frequently and are generally not associated with outbreaks of disease.

Both T. circumcincta and T. vitrinus are oviparous and follow the direct life-cycle typical of the trichostrongylids (Figure 1.2). Eggs are voided with the host faeces onto pasture where they hatch to release first-stage larvae (L1). These undergo subsequent moults to give second- and third-stage larvae (L2 and L3, respectively).
Under optimal conditions, (temperatures of 27°C), this process takes between 4-6 days but in northern Britain development is more likely to take 2 weeks. The survival of these preparasitic stages is highly dependant on the prevailing environmental conditions, particularly temperature and relative humidity. As *T. circumcincta* larvae are better able than most trichostrongylids to survive long
periods of cold, large numbers of *T. circumcincta* L₃ may overwinter on contaminated pasture in temperate climates (Boag and Thomas, 1977; Waller and Thomas, 1978). To complete the life-cycle, the infective L₃ require to be ingested by a suitable ruminant host. Within the host the parasite continues its development through two further moults before becoming adult, mating and commencing egg production.

*Teladorsagia (Ostertagia) circumcincta* (Stadelmann, 1894) Ransom, 1907, is a common parasite of the abomasum of sheep and goats. The development of this parasite following single infection has been previously described by Armour, Jarrett and Jennings (1966). By day 4 post-infection (PI) the L₃ have exsheathed in the rumen and penetrated the gastric glands of the abomasal mucosa. Here the larvae undergo a third and fourth moult, passing through the early, mid and late fourth larval stages (L₄) and emerging from the mucosa as immature adults by day 8 PI. Approximately 18-21 days PI the worms become adult and egg production may commence. The adult worms, measuring an average 7.5-8.5 mm for males and 9.5-12.2 mm for females, are haematophagic lumen dwellers.

However under certain conditions the infrapopulation dynamics may follow an alternative path. Larvae ingested towards the end of autumn are likely to cease their development and remain in the gastric glands as early fourth-stage larvae. This arrested development is termed 'hypobiosis' and occurs at a precise point in larval development. Hypobiosis may be influenced by host immunity to infection but seems to be more closely associated with environmental factors such as the declining autumn temperatures. Armour and Bruce (1974) have linked the hypobiosis of *T. circumcincta* in temperate climates to direct temperature effects on the metabolism
of the free-living stages. Developmental arrest is not permanent and larval
development resumes with improving environmental conditions. In experimental
studies using naïve challenge controls, retarded and/or arrested development may be
induced by immunity acquired after exposure to infection (Seaton, Jackson, Smith
and Angus, 1989b).

*Trichostrongylus vitrinus* (Looss, 1905) is a small nematode, (males 4-5.5
mm and females 5-6.5 mm long), which is common in the proximal third of the
small intestine of sheep and goats. The development of this parasite has been
described by Taylor (1977). Infective L\textsubscript{3} are ingested by the host and by the second
day PI the majority of these will have moulted to the L\textsubscript{4} stage. The larvae undergo a
further moult to reach the fifth or early adult stage 8-10 days PI. By day 17 PI the
worms become adult and egg production usually commences within 3 weeks of
infection (Coop, Angus and Sykes, 1979). Adult *T. vitrinus* burrow sub-epithelially
in the anterior small intestine and are non-haematophagous.

In common with *T. circumcincta*, *T. vitrinus* has the capacity to undergo
inhibited development within the host. However *T. vitrinus* larvae arrest their
development at the L\textsubscript{3} rather than the L\textsubscript{4} stage (Eysker, 1978; Waller, Donald and
Dobson, 1981). Eysker (1978) suggests that under northern European conditions
inhibition of *T. vitrinus* larvae is more likely to be driven by host immunity than
environmental conditions.

The epidemiology of small ruminant nematodiasis has been defined in terms
of parasite population dynamics (Gordon, 1949). The factors influencing nematode
epidemiology are complex (Figure 1.3) and have been reviewed by Armour (1980).
The population dynamics of *T. circumcincta* and *T. vitrinus* are typical of the
trichostrongylids with vertical transmission of infection. However the epidemiology of *N. battus* is very different in that transmission of infection is almost entirely lateral and inter-seasonal from one lamb generation to the next. Previous studies have demonstrated a succession of nematode species in grazing lambs (Crofton, 1955, 1957; Reid and Armour, 1972; Boag and Thomas, 1977). There are normally two waves of *T. circumcincta* infection on contaminated pasture (Boag and Thomas, 1977; Waller and Thomas, 1978). The first in late spring/early summer is due to the presence of large numbers of overwintering and hypobiotic larvae. Numbers of *T. circumcincta* larvae decrease during the summer but increase again in late summer/early autumn. Prediction models have suggested that this second wave may contain a high proportion of lamb-derived larvae (Paton, Thomas and Waller, 1984). Epidemiological studies of *T. vitrinus* have found evidence of only a single wave of infection in late summer/early autumn (Boag and Thomas, 1977). Studies have shown *T. vitrinus* to have only a limited ability to overwinter on pastures in Scotland (Jackson, 1982) and south-east England (Rose and Small, 1984) and these larvae may give rise to the small numbers of *T. vitrinus* seen in lambs in early summer.

1.1.3 Pathology

The effects of trichostrongylidosis on the host, and in particular young or immunocompromised animals, can be severe with a considerable risk of host mortality especially following the emergence of large numbers of hypobiotic larvae in early spring.
Infective *T. circumcincta* L$_3$ invade the abomasal parietal glands forming lesions. The presence of developing larvae causes destruction of the mature, differentiated (mucous, zymogenic and parietal) cells which are replaced with undifferentiated mucus-secreting cells (Armour *et al.*, 1966). This rapid cell division leads to mucosal cell hyperplasia and a thickening of the abomasal mucosa which is responsible for the clinical signs of infection. These include an increase in abomasal pH from 2.5 to 5 or even 7 as the HCl-producing parietal cells are replaced by functionally inactive cells, leakage of plasma proteins and hypoalbuminaemia (McKellar, 1993). The effect of this in heavy infections is to produce a catarrhal gastro-enteritis with associated diarrhoea, dehydration, anaemia, inappetence and subsequent weight loss. Due to the diarrhoea, the wool around the breach can
become heavily soiled (dags) predisposing the animal to further infestation, such as fly-strike.

Many of the sequelae of *T. vitrinus* infection are essentially similar to those described above. The presence of the adult worms may cause villous atrophy, flattening of the mucosa and erosion of the intestinal epithelium (Taylor and Pearson, 1979 a, b; Jackson, Angus and Coop, 1983). The integrity of the mucosa may be impaired as a consequence of the infiltration and activity of the infected site by inflammatory cells which in turn may lead to plasma protein loss and hypoalbuminaemia.

1.2 Factors influencing the parasite population

In natural infections the frequency of gastrointestinal nematode infection follows a negative binomial distribution describing a state of overdispersion (Barger, 1985). The result of this is that a relatively small proportion of the host population harbours a large proportion of the parasite population. Riffkin (1988) has estimated that the most resistant 50% of grazing animals may produce less than 10% of the worm eggs counted, whereas the most susceptible 15% of the flock may be responsible for over 50% of the egg output. The existence of such overdispersed parasite populations illustrates the importance of host immunity to infection.

1.2.1 Development and expression of acquired immunity

Host immunity to infection can be expressed in one of two ways; innate and acquired. Innate immunity is a non-specific and pre-existing phenomenon which provides a measure of the host’s ability to regulate parasite establishment, development, persistence and fecundity. In contrast, acquired immunity is highly
specific for a particular pathogen and is an active, adaptive and aggressive response which improves with repeated exposure to the same pathogen. Although acquired immunity may be the more important element in the host response to parasite infection, laboratory studies have shown that innate immunity plays an important role in the immune responses of mice infected with *Nematospiroides dubius* (Brindley and Dobson, 1981, 1983a).

Young animals are most susceptible to gastrointestinal nematode infection. In temperate regions, where grazing animals are exposed to continuous trichostrongylid infection, these animals are gradually able to acquire an immunity to subsequent infection. The development and expression of acquired immunity is a complex process (Figure 1.3) which may be strongly influenced by a number of factors, most notably the plane of host nutrition (Wagland, Steel, Windon and Dineen, 1984), the level of exposure to infection (Gill, 1991), the age of the host (Manton, Peacock, Poynter, Silverman and Terry, 1962; Chiejina and Sewell, 1974; Douch, 1988) and animal management (Cabaret, Anjorand and Leclerc, 1989).

Empirical studies on the effects of feed quality have emphasised the importance of protein intake for the development and expression of immunity towards gastrointestinal nematodes in sheep (Wagland *et al.*, 1984; Abbott, Parkins and Holmes, 1985; Abbott and Holmes, 1990; van Houtert, Barger, Steel, Windon and Emery, 1995) and goats (Blackburn, Rocha, Figueiredo, Berne, Vieira, Cavalcante and Rosa, 1991). The importance of protein intake has been supported by laboratory studies in which the expulsion of *Nippostrongylus brasiliensis* from protein-deficient rats was impaired (Bolin, Davis, Cummins, Duncombe and Kelly, 1977; Cummins, Duncombe, Bolin, Davis and Yong, 1987). However, nutrition may
be more important in counteracting the effects of parasite infection than preventing worm establishment (Blackburn et al., 1991).

Previous exposure to gastrointestinal nematode infection may be the single most important factor in the development of acquired immunity. Studies conducted by Gray, Albers and Piper (1990) have suggested that powerful environmental influences acting on very young lambs may have important consequences for the expression of immunity to infection some 3-5 months later.

Young lambs are less able to effectively regulate their nematode burdens than are older animals (Gibson, Parfitt and Everett, 1970; Gregg, Dineen, Rothwell and Kelly, 1978). Generally, immunity to gastrointestinal nematode infection in sheep gradually improves with age over the first 12 months (Watson and Gill, 1991). This has been reported for sheep infected with *H. contortus* (Manton et al., 1962; Benitez-Usher, Armour, Duncan, Urquhart and Gettinby, 1977), *T. colubriformis* (Gibson and Parfitt, 1972, 1973; Dineen, Gregg and Lascelles, 1978) and *T. circumcincta* (Smith, Jackson, Jackson and Williams, 1985). The relative immunological unresponsiveness of immature lambs has been described by Watson, Colditz, Andrew, Gill and Altmann (1994) and supports other studies which suggest that lambs are unable to mount an effective immunity to nematode infection until they are at least 6 months of age (Waller and Thomas, 1981; Soulsby, 1985). It has been suggested that the unresponsiveness of young lambs may be partly due to the immaturity of gut effector mechanisms rather than the failure to produce parasite-specific antibodies (Gregg et al., 1978). The factors involved in the development of age-related immunity are as yet unknown, but have been linked to factors such as puberty, bodyweight and condition rather than chronological age (Abbott and Holmes, 1990).
The time required for the acquisition of immunity in lambs is dependent on
the presence of a moderate level of infection (Barger, 1988a). Thus regular and
highly efficacious anthelmintic treatments may actually curb the development of host
immunity (Gibson et al., 1970; Luffau, Vu Tien Khang, Bouix, Nguyen, Cullen and
Ricordeau, 1990). Studies have shown that Merino wethers can acquire immunity to
*H. contortus* (Barger, Le Jambre, Georgi and Davies, 1985) and *T. colubriformis*
(Dobson, Waller and Donald, 1990) infections after 7 weeks of continuous
challenge. Similarly, studies conducted at Moredun using Greyface cross Suffolk
lambs challenged daily with either *T. circumcincta* (Seaton et al., 1989b) or *T.
vitrinus* (Seaton, Jackson, Smith and Angus, 1989a) showed that a partial immunity
to larval establishment was expressed after 4 weeks. By 8-12 weeks after first
infection the lambs exhibited almost complete immunity to incoming larvae.

Acquired immunity to gastrointestinal nematode infection may be expressed
in a number of stages (Rothwell, 1989). After an initial period of high susceptibility
to infection the first manifestations of acquired immunity are a failure of incoming
larvae to establish, and a retardation in the development of established worms
(Seaton et al., 1989a, b). In the later stages of immunity established adult parasites
may be expelled. This can be a sudden loss as exhibited by the "self-cure"
phenomenon in *H. contortus* infected sheep (Stewart, 1953), or as a result of steady
density-dependent losses as seen in *T. circumcincta* infections (Waller and Thomas,
1978). Effector mechanisms that have been observed in the development of
immunity against *T. colubriformis* are lowered female worm fecundity (Gibson et al.,
1970; Gibson and Parfitt, 1972, 1973; Dineen and Windon, 1980; Wagland et al.,
1984) and a decrease in the ratio of male to female adult worms (Dineen and
Windon, 1980). Similar effects have been reported in the development of responsiveness to other gastrointestinal nematodes (Presson, Gray and Burgess, 1988; Gill, Gray and Watson, 1991).

Small ruminants not only have the ability to regulate worm populations (resistance) but may also have an ability to tolerate worm burdens (resilience). Resistance to infection is an immunological characteristic which has been defined as "the ability to suppress establishment and/or subsequent development of infection" (Albers, Burgess, Adams, Barker, Le Jambre and Piper, 1984). However, the producer's aim is always to attain the maximum efficiency of production, and this can be measured through host resilience. Albers et al. (1984) have suggested that resilience can be defined as "the ability to maintain a relatively undepressed production level when infected." Studies examining the responses of Merino weaners artificially challenged with 11,000 *H. contortus* larvae have demonstrated that there is a strong positive correlation (0.56) between resistance and resilience (Albers et al., 1984). Recent New Zealand studies with Romney sheep exposed to naturally acquired nematode infection have investigated the feasibility of using resilience rather than resistance as a potential breeding option (Morris, Watson, Bisset, Vlassoff, Douch, Gray, Woolaston and Eaton, 1995). The authors reported low heritability estimates for resilience (0.06 to 0.14) suggesting that progress in a selective breeding programme would be slow. There was no significant association between individual resilience and faecal egg count (FEC) following nematode infection. However it is not clear at present how resilience is expressed under field conditions (Albers and Gray, 1986).
Traditionally the parasitology of goats has been assumed to be very similar to that of sheep. Thus there has been little information gathered regarding the development of immunity in goats. One reason for this is that the vast majority of goats are to be found in Third World countries where they supply the local community and have little commercial importance. However, as the economic importance of goats has risen, increased research has been conducted which suggests that goats may differ greatly from sheep in their responses to gastrointestinal nematode infection (Pomroy, 1985).

Previous studies have shown that when sheep and goats are grazed together on contaminated pasture, goats are significantly more susceptible to nematode infection as measured both by faecal egg counts and subsequent worm burdens (Le Jambre and Royal, 1976; Anon, 1982; Brunsdon, 1983; Le Jambre, 1984a; Pomroy, Lambert and Betteridge, 1986; Watson and Hosking, 1989; Jallow, McGregor, Anderson and Holmes, 1994). This increased susceptibility is expressed at all ages, and goats differ greatly from sheep in remaining highly susceptible to trichostrongylid infection when adult. A number of studies, predominately in Australia and New Zealand, have investigated the development of age- and experience-related immunity in goats. The results appear inconclusive (McKenna, 1984; Pomroy, 1985). Several have reported an absence of any evidence for development in host immunity (Brunsdon, 1983; Kettle, Vlassoff, Reid and Horton, 1983; Pomroy and Charleston, 1989b; Watson and Hosking, 1989; Woolaston, Singh, Tabunakawai, Le Jambre, Banks and Barger, 1992b). Others have suggested that goats may develop some degree of responsiveness, but that this is less effective,
slower or later starting than that of sheep (Pomroy et al., 1986; Pomroy and Charleston, 1989a).

The reasons for the observed differences in acquisition of immunity are unclear. Le Jambre and Royal (1976) have postulated that grazing behaviour may play an important role. Goats are preferential browsers (French, 1970) and if feeding on scrub higher than 12.5 cm above the ground would avoid over 99% of infective nematode larvae (Silangwa and Todd, 1964). Due to their browsing habit, goats may not have been under pressure to evolve as efficient an acquired immunity to gastrointestinal nematodiasis as have grazing sheep. Indeed goats appear to be much more susceptible to stocking rate effects than are sheep (Le Jambre, 1984a). Studies of sheep and goat grazing behaviour have shown that when forced to graze together with sheep, goats have a much higher larval intake (Jallow et al., 1994), which may serve to exaggerate the effects of increased susceptibility. However, under tropical conditions where goats are able to browse freely, they may exhibit lower faecal egg counts than grazing sheep (Vercruysse, 1983). Though more susceptible to nematode infection, there is evidence to suggest that goats are more tolerant (resilient) of the effects of infection than are sheep (Brunsdon, 1983).

1.2.2 Breed differences

Within species there is considerable evidence of breed or strain related differences in immunity to parasite infection (Gray, 1991). Variation has been reported between cattle breeds in their responsiveness to tick and Trypanosoma infection (Turner and Short, 1972; Murray, Clifford, Gettinby, Snow and McIntyre, 1981). Breed differences in acquired immunity in sheep to nematodiasis were first reported by Stewart, Miller and Douglas (1937). Since then breed variation has been
recorded from sheep infected with *H. contortus* and *T. circumcincta* (Scrivner, 1964; Loggins, Swanson and Koger, 1965; Jilek and Bradley, 1969; Rhadakrishnan, Bradley and Loggins, 1972; Bradley, Rhadakrishnan, Patil-Kulkarni and Loggins, 1973; Altaif and Dargie, 1978b, c; Preston and Allonby, 1978, 1979a; Courtney, Parker, McClure and Herd, 1984; 1985a, b) and to a lesser extent *Trichostrongylus* spp. (Ross, 1970). Preston and Allonby (1978) have shown that goats exhibit breed differences in immunity to *H. contortus*. Breed differences have also been documented between mouse strains in their ability to respond to *Trichinella spiralis* and *Heligmosomoides polygyrus* infection (Wakelin and Donachie, 1983; Lawrence and Pritchard, 1994).

1.2.3 Genotype

There is also considerable variation between individuals in outbred populations in their ability to resist parasite infection (Wakelin, 1978). This within-breed variability may often be as great as that seen between breeds (Gray, Presson, Albers, Le Jambre, Piper and Barker, 1987). Within-breed variation was first recorded by Clunies Ross (1932). Since then genetic variation in resistance has been reported within a number of sheep breeds (including Merino, Dorset, Romney and Corriedale breeds) to infection with *H. contortus* (Warwick, Berry, Turk and Morgan, 1949; Whitlock, 1958; Le Jambre, 1978; Albers, Gray, Piper, Barker, Le Jambre and Barger, 1987; Gray et al., 1987; Piper, 1987), *T. colubriformis* (Gibson and Parfitt, 1972, 1973; Chiejina and Sewell, 1974; Windon and Dineen, 1984), *T. circumcincta* (Scrivner, 1967) and mixed infection with *H. contortus*, *T. colubriformis*, *T. circumcincta* and *Nematodirus* spp. (Piper, Le Jambre, Southcott and Ch’ang, 1978; Watson, Baker and Harvey, 1986). Studies with *H. contortus*
infection in Kenya (Preston and Allonby, 1978) and gastrointestinal strongyles in France (Richard, Cabaret and Cabourg, 1990) have demonstrated that goats also exhibit genetic variation in responsiveness. In contrast, the authors of a recent caprine study in Fiji found little evidence for variation in resistance to mixed *T. colubriformis* and *H. contortus* infection (Woolaston *et al.*, 1992b). Variation in resistance has also been recorded within cattle breeds exposed to mixed nematode infection (Stear, Nicholas, Brown, Tierney and Rudder, 1984).

1.2.4 Sex

The sex of the host may have an important influence on the development and expression of immunity to infection (Barger, 1993a). The general findings from ruminant and laboratory studies are that entire males are more susceptible to nematode infection than are females or castrated males. Male sheep have been shown to be more susceptible than females to experimental infections with *Oesophagostomum columbianum* (Dobson, 1964; Bawden, 1969), *T. colubriformis* (Windon and Dineen, 1981), *H. contortus* (Colglazier, Lindahl, Wilson, Whitmore and Wilson, 1968; Luffau, Péry and Charley, 1981; Adams, 1989) and *T. circumcincta* (Gruner, Mandonnet, Bouix, Vu Tien Khang, Cabaret, Hoste, Kerboeuf and Barnouin, 1994; Stear, Bairden, Duncan, Gettinby, McKellar, Murray and Wallace, 1995). Other studies have found no evidence of sex-related differences in susceptibility to mixed nematode infection (Albers *et al.*, 1987; Woolaston, Barger and Piper, 1990) although the animals used in these latter studies may have been pre-pubertal. Increased male susceptibility has also been reported from studies of *N. dubius* infections in mice (Dobson, 1961a) and rats (Dobson, 1961b) and *N. brasiliensis* in rats (Waddell, Jarrett and Murray, 1971), mice (Swanson, Falvo and
Bone, 1984) and hamsters (Solomon, 1966). These laboratory studies have suggested that the greater susceptibility of entire males may be due to testicular hormones decreasing male resistance (Solomon, 1966; Waddell et al., 1971), to ovarian hormones increasing female resistance (Dobson, 1961a, b) or both (Swanson et al., 1984).

1.2.5 Reproductive status

A periparturient relaxation in immunity during pregnancy and lactation has been observed in a number of species including rabbits (Dunsmore, 1966), guinea pigs (Connan, 1968), rats (Connan, 1970), mice (Ngwenya, 1977), sheep (Lloyd, 1983a) and recently goats (Rahman and Collins, 1992). It has been suggested that relaxation may be due to the suppression of T-cell dependent immune responses which in turn impairs the production of antibodies to T-cell dependent antigens (Lloyd, 1983b). This relaxation in immunity is thought to affect a wide variety of effector mechanisms including those responsible for maintaining larval hypobiosis, adult fecundity and mortality, and control of larval establishment and development (Gibbs and Barger, 1986). A similar periparturient rise (PPR) in faecal egg count has been recorded following protostrongylid infection in ewes (Cabaret, Dakkak and Bahaida, 1980). In contrast, Jeffcoate, Fishwick, Bairden, Armour and Holmes (1990) were unable to detect any loss in cell-mediated or humoral immunity in periparturient ewes exposed to infection with T. circumcincta.

During the PPR all of the parasitological manifestations of acquired immunity, most notably resistance to larval establishment, arrested larval development, lowered worm fecundity and expulsion of established worms, appear to be compromised. The relaxation of immunity has been associated with lactation
rather than pregnancy as studies have shown the phenomenon to be abolished by removal of the suckling young (O’Sullivan and Donald, 1973). Although the cause of the PPR is unknown early studies have implicated the hormone prolactin (Barger, 1993a). However several studies have suggested that though prolactin may play a part in maintaining the PPR it is not itself responsible for initiating the response (Coop, Mellor, Jackson, Jackson, Flint and Vernon, 1990; Jeffcoate et al., 1990). In the former study the authors showed that inducing high prolactin levels in unbred ewes did not increase their susceptibility to *T. circumcincta* infection. The authors of the latter study treated *T. circumcincta*-challenged, lactating ewes with bromocryptine which significantly reduced plasma prolactin concentrations, but had little influence on the periparturient rise in FEC (Jeffcoate et al., 1990).

Interestingly, there is evidence that the loss of immunity to infection may be species-specific. Brunsdon (1970) noted that grazing lactating ewes showed increased susceptibility to infection with *Haemonchus* and *Ostertagia* but not *Nematodirus* or *Trichostrongylus*. This was reversed in a study by O’Sullivan and Donald (1973) which reported the increased susceptibility of ewes to *T. colubriformis* but not *H. contortus* infections. A similar result was recorded by Gibbs and Barger (1986) in which lactating ewes were found to be more susceptible to new infections with *T. circumcincta* and *T. colubriformis* but were as resistant to *H. contortus* infection as were dry ewes. Jackson, Jackson and Williams (1988a) have reported a loss of acquired immunity towards infection with *T. circumcincta* but not *T. vitrinus* in pre-periparturient ewes.
1.3 Control of gastrointestinal nematodoses

The aim of all nematode control strategies is to reduce host/parasite contact to levels at which there is no detrimental effect on animal production (Brunsdon, 1980). Attempts at control can be directed towards one of two distinct but very closely related parasite populations; the ‘suprapopulation’ consisting of all stages of the parasite species within the ecosystem, or the ‘infrapopulation’, those parasites within an individual (Esch, Gibbons and Bourque, 1975). In temperate climates where large numbers of nematode eggs and larvae may overwinter on pasture, the suprapopulation normally constitutes by far the majority of the total parasite population.

1.3.1 Chemotherapy

Chemotherapy is currently the most important weapon against gastrointestinal nematode infection in the animal producer’s armoury. Prior to the introduction of the first broad spectrum anthelmintics (thiabendazole in 1961) chemotherapeutic control was reliant upon relatively inefficient narrow spectrum (phenothiazine) or often dangerous drugs (carbon tetrachloride, organo-phosphorus compounds). Current chemotherapeutic control is based on safe and highly effective broad spectrum anthelmintic families; such as the benzimidazoles, imidazothiazoles and avermectins/milbemycins.

These broad spectrum drenches have commonly been used in one of two ways. Their oldest and simplest use is therapeutic in response to the symptoms of nematodosis. The obvious disadvantages of chemotherapy are that production losses are likely to have already occurred and the presence of large numbers of infective larvae on pasture is likely to result in rapid post-treatment reinfection. Today
treatment is most commonly prophylactic, where anthelmintic is administered before obvious signs of infection in an attempt to limit the size of the infrapopulation, thus reducing pasture contamination, the parasite suprapopulation and the risks of subsequent reinfection.

1.3.2 Anthelmintic resistance

Unfortunately the high dependence on chemoprophylaxis and chemotherapy to minimise the impact of gastrointestinal nematode infection has in turn led to the selection of nematodes which are resistant to those drugs. Resistance to one or more of the broad spectrum drugs is widespread among the gastrointestinal nematodes of sheep and goats (Prichard, Hall, Kelly, Martin and Donald, 1980; Waller and Prichard, 1986; Waller, 1987; Prichard, 1990; Taylor, 1990; Jackson, 1993; Waller, Echevarria, Eddi, Maciel, Nari and Hansen, 1996). Though the incidence of anthelmintic resistance among cattle nematodes is relatively low (Prichard, 1990) it is increasing and there are reports of resistance to oxfendazole (Eagleson and Bowie, 1986), levamisole (Geerts, Brandt, Kumar and Biesemans, 1987), morantel tartrate (Borgsteede, 1988) and more recently ivermectin (De Vaney, Craig and Rowe, 1992).

Anthelmintic resistance was first reported to phenothiazine in *H. contortus* recovered from goats by Drudge, Leland and Wyant (1957). Subsequently, resistance to the broad spectrum anthelmintics has been reported from field populations of *H. contortus*, *T. colubriformis* and *T. circumcincta* throughout the world. Resistance has also been reported towards narrow spectrum drugs such as the salicylanilides (Rolfe, Boray, Fitzgibbon, Parsons, Kemsley and Sangster, 1990). As the prevalence of resistance within one anthelmintic family increases, it has been common for
producers to switch to alternative families, often resulting in the emergence of multiple anthelmintic resistant nematode strains (van Wyk and Malan, 1988).

The problem appears to be most acute among the gastrointestinal nematodes of goats, with resistance to at least one of the broad spectrum families reported from Australia (Barton, Trainor, Urie, Pyman and Wolstencroft, 1985), New Zealand (Kettle et al., 1983; Scherrer, Pomroy and Charleston, 1989; Badger and McKenna, 1990; McKenna, Badger, McKinley and Taylor, 1990; Watson and Hosking, 1990), the USA (Craig and Miller, 1990), Brazil (Echevarria and Trindade, 1989) and France (Kerboeuf and Hubert, 1985). Studies conducted on grazing Scottish fibre goats have detected resistance among *T. circumcincta* towards benzimidazoles (Scott, Bairden, Holmes and McKellar, 1989; Jackson, Coop, Jackson, Scott and Russel, 1992a; Jackson, Jackson, Little, Coop and Russel, 1992b). The incidence of multiple anthelmintic resistance, (resistance to two or more different anthelmintic families), has been steadily increasing, particularly among goats in New Zealand (Kettle et al., 1983; McKenna et al., 1990; Watson and Hosking, 1990; Pomroy, Whelan, Alexander, West, Stafford, Adlington and Calder, 1992), and a strain of *T. circumcincta* exhibiting resistance to both benzimidazole and ivermectin has also been isolated from Scottish Cashmere goats (Jackson et al., 1992a).

The development of anthelmintic resistance is the result of the selection pressures exerted on the nematode infrapopulation by the use of highly efficacious drug treatments. It is assumed that resistance is a pre-adaptive phenomenon, the gene or genes which confer resistance being present at low frequencies in the population prior to exposure to the drug. There would appear to be three phases to the development of anthelmintic resistance (Prichard, 1990). Initially there is a period of
susceptibility as the frequency of resistant individuals within the population is very low. Continued exposure to the drug leads to increased selection and survival of heterozygous resistant individuals. The final result of the intense selection pressures imposed is the predominance of homozygous resistant individuals. As resistance is selected for most strongly when both heterozygous and homozygous individuals survive administration of anthelmintic, factors which reduce the efficacy of treatment, enabling survival of the former, are of greatest importance in the selection for and development of anthelmintic resistance. Low efficacy treatments have been shown to select rapidly for resistance in ovine strains of *Teladorsagia* (Martin, 1990).

The most important factors in the development of resistance are the efficacy and frequency of treatment. Suppressive regimes with treatments given close to the parasite’s prepatent period (usually around three weeks) impose the most intense selection pressure as only those individuals displaying some degree of resistance will make any contribution to the next generation. Similarly, treating and moving stock to clean pasture may be counterproductive as, again, survivors of anthelmintic treatment will make a major genetic contribution to the next parasite generation (Taylor and Hunt, 1989; Smith, 1990). Goats may play a disproportionately important role in the development of anthelmintic resistant nematodes. Several studies have shown that the pharmacokinetics of the levamisole and benzimidazole families differ between sheep and goats (Galtier, Escoula, Camguilhem and Alvinerie, 1981; Hall, Ritchie and McDonell, 1981; Kettle *et al.*, 1983; Gillham and Obendorf, 1985; McKenna and Watson, 1987; Sangster, Rickard, Hennessy, Steel and Collins, 1991; Hennessy, Sangster, Steel and Collins, 1993). The anti-parasitic
activity of levamisoles is dependent upon peak drug concentration, whereas that of the benzimidazoles is proportional to the duration of exposure (Barragry, 1984a, b). Anything which interferes with the mode of action may reduce the efficacy of that drug. Comparative studies have suggested that the high prevalence of oesophageal groove closure and rumen bypass following oral drenching in goats compared with sheep may contribute to reduced drug bioavailability and efficacy (Sangster et al., 1991). The increased metabolic activity of goats may result in significantly reduced peak blood levels and duration compared to sheep (Prichard and Hennessy, 1981; Gillham and Obendorf, 1985; Hennessy et al., 1993). It has been suggested that reduced drug efficacies in goats may enhance the selection and development of resistance (Gillham and Obendorf, 1985; Charles, Pompeu and Miranda, 1989) which could then be transferred to sheep (Kettle et al., 1983). If underdosing in goats is the problem, increasing the administered dose would appear to be the obvious solution; unfortunately this is not necessarily the case. Overdosing is undesirable not only in terms of levels of tissue residues, toxicity, in particular of levamisole (Galtier et al., 1981) and cost, but also has been shown to offer little or no improvement in drug bioavailability and efficacy in goats (Sangster et al., 1991).

The problems caused by anthelmintic resistance are compounded by the lack of any evidence to suggest that drug resistant populations will revert towards susceptibility in the absence of anthelmintic treatment. Studies conducted in Australia have suggested that *H. contortus* and *T. colubriformis* resistant to benzimidazole were unlikely to revert to practicable levels of susceptibility within 10 years of last exposure to the drug (Hall, Ritchie and Kelly, 1982). In Europe no reversion to benzimidazole susceptibility was evident in *H. contortus* after six years
of levamisole treatment in one study (Borgsteede and Duyn, 1989), and after nine years of alternate ivermectin and levamisole treatment in another (Jackson, unpublished data).

1.3.3 Anthelmintic resistance and chemical control strategies

Anthelmintics are likely to remain the mainstay of nematode control schemes for the foreseeable future. Due to the difficulties inherent in bringing new anthelmintics onto the market (Hotson, 1985) it is important that existing drugs are utilised more effectively. To this end nematode control programmes (such as “Wormkill” and “Drenchplan”) have been introduced in Australia with the aim of preserving the efficacy of the currently available anthelmintics through reduced treatment frequency, avoiding underdosing and rotational use of drug families (Waller, 1987). In the short term it may be possible to extend the useful life of current anthelmintics through manipulation of their pharmacokinetics. A benzimidazole/levamisole combination has been shown to give a synergistic increase in efficacy against *H. contortus* in sheep (Bennet, Behm, Bryant and Chevis, 1980). Modifications to the benzimidazole molecule have resulted in improved solubility and enabled its delivery as an injection (Hennessy, Lacey and Prichard, 1983). This together with alterations to the metabolism and elimination of the drug have led to increases in bioavailability and efficacy (Hennessy, Lacey, Prichard and Steel, 1985). Controlled release devices or boluses which extend the time the parasite is in contact with the anthelmintic may overcome the existing problems associated with rapid drug clearance following oral drenching. Whether or not sustained release boluses will accelerate selection for resistance has been in dispute (Anderson, 1985a, b) though field studies have shown that albendazole boluses may, initially, provide
effective control of sheep nematodes which had displayed resistance to oral benzimidazole treatment (Barger, 1988b).

As the action of many drugs depends on the length of time that they are in contact with the parasite drug efficacy may be enhanced by prolonging this contact. Extending the period of drug bioavailability through splitting the anthelmintic dose has been shown to increase the efficacy of the benzimidazoles. Bogan, Benoit and Delatour (1987) have shown that multiple dosing with 3 oxfendazole treatments at 24 hour intervals was more effective against naturally acquired T. circumcincta infections in goats than administering the same total volume of drug in a single dose. Similarly, Sangster et al. (1991) reported an increase in oxfendazole efficacy against natural H. contortus, Ostertagia spp. and Trichostrongylus spp. infection in goats when the dose was given as 2 treatments 12 hours apart. Altering feed intake has also been shown to enhance the efficacy of the benzimidazoles. Halving the feed intake of sheep for 36 hours prior to and after oxfendazole treatment reduced digesta flow and led to increased drug bioavailability (Ali and Hennessy, 1993). Drug efficacy against benzimidazole resistant H. contortus and T. colubriformis was significantly higher in those animals with a reduced feed intake. Ali and Hennessy (1995b) suggest that in the field situation, penning sheep and withholding or reducing feed for 12-24 hours before and after anthelmintic treatment would give significant improvements in drug efficacy.

1.4 Alternative control strategies

The increasing prevalence of anthelmintic resistance poses many problems for all those involved in small ruminant production world-wide. This, together with
the very high costs and stringent regulations required for the discovery, development and registration of new drugs (Hotson, 1985) stress the importance of preserving the effectiveness of the existing anthelmintics. In addition, increasing awareness of environmental issues and consumer demand for animal products and pastures free from chemical residues are intensifying the need for producers to seek alternatives to chemotherapeutic control. There are three main areas of research into alternative control strategies; managemental, biological and immunological.

4.1 Managemental control strategies

The greatest benefits to control are achieved when drug treatment is combined with some form of grazing management whereby treated animals are moved to pastures with lower levels of parasite contamination. However, the results of field studies disagree as to whether this ‘treat-and-move’ strategy promotes the development of anthelmintic resistance (Martin, Anderson and Jarrett, 1985) or has no greater effect than treating sheep which are set-stocked on the same pasture (Waller, Donald, Dobson, Lacey, Hennessy, Allerton and Prichard, 1989). The premise behind most integrated grazing management programmes is to move susceptible animals off contaminated pastures before the risk of infection becomes too high. Animals are then moved to pastures with much lower levels of contamination. These ‘safe’ pastures are the result of a period of little or no parasite contamination. As simply resting the pasture is seldom a viable option, the pasture can be used for crop production or to graze resistant adults or individuals of another species. Alternate grazing with cattle and sheep has been proposed for the control of bovine parasitic gastro-enteritis, though a four year study conducted in Scotland found this to be unsuccessful (Bairden, Armour and Duncan, 1995). The most
important factors in determining the success of integrated grazing programmes are the host-specificity of the parasite, the longevity of the free-living stages and the duration of pasture resting or alternate use.

1.4.2 Biological control strategies

Biological control strategies follow one of two approaches both of which are directed towards the parasite suprapopulation; genetic manipulation of the parasite or the use of nematophagous fungi and other micro-organisms. Studies in Australia have attempted to control the level of *H. contortus* infection in sheep through hybridisation of this species with its bovine counterpart, *Haemonchus placei* (Le Jambre, 1984b). The progeny of this mating are sterile and may be able to exclude normal *H. contortus* under laboratory conditions. Unfortunately this has yet to be demonstrated under field conditions. A different approach has been taken by workers in South Africa who have attempted to reintroduce susceptible genes into the *H. contortus* suprapopulation (van Wyk, 1990). Although the study found this strategy to be successful, it is less likely to be as beneficial in temperate climates due to differences in parasite suprapopulation dynamics and the low fecundity of the more important resistant species such as *Teladorsagia* spp.

Biological control using micro-organisms has received relatively little attention. It is known that there are a number of bacteria, protozoa, viruses and fungi which will infect or prey on the free-living stages of nematodes, and it is possible that these may provide a useful addition to the armoury of control measures (Waller, 1992; Grønvold, Wolstrup, Nansen, Henriksen, Larsen and Bresciani, 1993; Waller and Larsen, 1993). Field studies have suggested that the microfungus *Duddingtonia flagrans* may be effective against the nematode larvae of cattle and horses on pasture.
1.4.3 Immunological control strategies

Immunological control strategies utilise, and attempt to enhance, the host’s responses to gastrointestinal nematode infection. There are two approaches to immunological control; the development of vaccines and the genetic selection of individuals with increased levels of resistance to infection.

Considerable research has been directed towards the development of protective vaccines against gastrointestinal nematodes (Clegg and Smith, 1978; Lloyd, 1981; Miller, 1987a; Tavernor, Smith, Langford, Graham and Munn, 1992a; Tavernor, Smith, Langford, Munn and Graham, 1992b; Turnbull, Bowles, Wiltshire, Brandon and Meeusen, 1992). However, on the whole the results have been disappointing. Although successful levels of protective immunity have been produced in laboratory studies (MacKenzie, Jungery, Taylor and Ogilvie, 1980; Smithers, Hackett, Ali and Simpson, 1989) and against *Ancylostoma caninum* infection in dogs (Miller, 1978) only one vaccine, using radiation-attenuated larvae, against *Dictyocaulus viviparus* infection in cattle has been commercially viable (Peacock and Poynter, 1980). Recent studies with young lambs have demonstrated varying levels of protection to artificial *H. contortus* infection following vaccination with larval antigens (Tavernor *et al.*, 1992a, b; Turnbull *et al.*, 1992). As computer modelling studies have suggested that a vaccine could have an appreciable epidemiological impact without being particularly efficient, the future for vaccine development may be quite bright (Barger, 1993b).
One of the most promising options for future nematode control is the genetic manipulation of the host to produce individuals exhibiting increased levels of resistance to or resilience of infection. Immunological function has been shown to be under genetic control (Wakelin, 1985) and the existence of both between- (Stewart et al., 1937) and within-breed (Whitlock, 1958) variation in immunological responsiveness to gastrointestinal nematode infection has been known for some time. The high degree of within-breed variation makes the implementation of a selective breeding programme a feasible option (Gray et al., 1987; Le Jambre, 1978).

1.5 Selective breeding programmes

Building on the within-breed differences in responsiveness, successful selective breeding programmes have been initiated in Australia and New Zealand producing lines of sheep with increased levels of resistance to infection with *H. contortus*, *T. colubriformis* and *T. circumcincta* (see reviews by Albers et al., 1987; Piper, 1987; Baker, Watson, Bisset and Vlassoff, 1990; Windon, 1990; Gray, 1991).

1.5.1 Trichostrongylus selection lines

A programme to produce lines of Merino sheep either resistant (high responders) or susceptible (low responders) to *T. colubriformis* infection was established in Australia in 1976. The parent generation were selected on the basis of their responsiveness as lambs to vaccination with irradiated *T. colubriformis* larvae (Windon, Dineen and Kelly, 1980). Since then the immunological status of lambs has been determined following vaccination at 8 and 12 weeks of age with 20,000 irradiated *T. colubriformis* larvae, anthelmintic treatment at 16 weeks and homologous challenge with 20,000 normal *T. colubriformis* one week later (Dineen
These studies were conducted on worm-free female and castrated male lambs under pen conditions in an attempt to eliminate environmental influences. Responsiveness was determined using the mean of five faecal egg counts taken at two-week intervals commencing three weeks post-challenge. Within each generation lambs from the high responder line had significantly lower egg counts than did low responders. In addition, within each line, female lambs were considerably more immunologically responsive than males (Windon and Dineen, 1981; Windon et al., 1987). The most rapid improvement in responsiveness was seen in the first generation with more modest advances in later generations. Heritability, indicating the proportion of variation between individuals which is under genetic control, was estimated to be 0.39 (± 0.27) for pooled male and female egg count data (Windon et al., 1987). Heritabilities were not calculated separately for wether males and females. After approximately ten years of selection, sheep from the high responder line display high enough levels of resistance to infection that anthelmintic treatments may be largely unnecessary (Barger, 1993b). This observation has been supported by computer simulation studies using the Barnes and Dobson (1990) model (Barger, 1989; Windon, 1990).

1.5.2 Haemonchus selection lines

Two Merino selection programmes for increased resistance to *H. contortus* infection have been established in Australia. The first of these, begun in 1977, has established divergent lines of Merinos exhibiting either increased or decreased resistance to *H. contortus* infection and has been described by Piper (1987) and Woolaston (1990). Responsiveness was determined on the basis of maximum FEC of
5-6 months-old grazing lambs following artificial challenge with 10,000 *H. contortus* larvae. Unlike the *Trichostrongylus* selection programme, little response to selection was apparent over the first five years. Since that time significant between-line differences in responsiveness have been recorded (Piper, 1987; Woolaston, 1990). Heritability to *H. contortus* in this line has been estimated at 0.33 (± 0.03) (Windon, 1990).

The second *H. contortus* selection programme (also in Australia) was set up in 1981 to examine the resistance and resilience of young Merinos infected with *H. contortus* (Albers et al., 1984; Albers and Gray, 1986; Albers et al., 1987). These parameters were measured in 4-5 months-old grazing Merino lambs which were given an additional artificial challenge of 11,000 infective *H. contortus* larvae. Resilience was found to have a heritability no greater than zero though the heritability of resistance, as measured by FEC four weeks after artificial challenge, was estimated to be 0.34 (± 0.10) (Albers et al., 1987).

Studies in Hungary have selected high and low responder Merinos following double artificial *H. contortus* infection. The lambs were raised worm-free before primary challenge at around six months of age. Responsiveness was determined using FECs following secondary challenge. The heritability of responsiveness to *H. contortus* infection was estimated to be 0.49 (± 0.17) (Sréter, Kassai and Takács, 1994).

1.5.3 *Selection based on naturally acquired field infections*

Selection programmes in New Zealand differ from those in Australia in the relative importance of the parasite species and in challenge protocol. In the drier areas of Australia *H. contortus* and *T. colubriformis* are the most important sources
of nematodiasis whereas in New Zealand, as in other temperate climates, *Teladorsagia* (Ostertagia) spp. and *Trichostrongylus* spp. predominate. Australian studies have used artificial challenge with known numbers of infective larvae, whereas New Zealand studies have relied on naturally acquired field infections, sheep being exposed to a number of nematode species. Romney lambs are weaned at around 3 months of age, treated with anthelmintic and allowed to graze contaminated pasture. Responsiveness is measured using two FECs (the first taken in late summer, the second approximately six weeks later) separated by an anthelmintic treatment (Baker et al., 1990). It has been suggested that the sample taken post-treatment may be the most informative as it reflects the ability of the host to regulate worm establishment and development (Baker et al., 1990). The heritability estimates recorded for FEC in these studies are very similar to those calculated for Australian studies at around 0.3-0.4 (Baker, Watson, Bisset, Vlassoff and Douch, 1991).

In Australia, Riffkin and Yong (1984) and Riffkin (1988) have been selecting Merinos for increased resistance to natural pasture infection with *Teladorsagia* spp. and *Trichostrongylus* spp. using both FEC and lymphocyte responsiveness to parasite antigen. Detailed results from these studies have not been published.

1.5.4 Correlations with productivity

It has been demonstrated that resistance to nematode infection in sheep as measured by FEC is moderately heritable, with estimates of about 0.35 in naturally infected Romneys (Baker et al., 1991) and 0.30 for a single FEC from Merinos following artificial challenge (Woolaston, Windon and Gray, 1991). It is important to bear in mind that these estimates are very similar to those obtained for production
traits such as milk yield, fleece weight and body weight which have been selected for successfully in the past.

The greatest incentives for producers to take onboard selective breeding programmes are not marginal economic gains in productivity, but rather the potential to avoid the catastrophic losses which may occur in the absence of effective anthelmintic treatment. Previous studies have suggested that there is no correlation between the resistance status of the host and production parameters such as wool quality, fleece weight and live-weight gain when animals are uninfected, but that more responsive individuals can maintain higher levels of productivity in the presence of nematode infection (Albers et al., 1987; Piper and Barger, 1988; Woolaston, 1990). Recent studies in New Zealand have shown that Romney sheep which have been selected for increased fleece weight may be more susceptible to nematode infection than are control animals though they appear to be more resilient to the effects of parasitism (Howse, Blair, Garrick and Pomroy, 1992; Williamson, Blair, Garrick, Pomroy and Douch, 1994).

New Zealand studies have shown faecal consistency in grazing Romney sheep to be negatively correlated with faecal egg counts, more liquid faeces being associated with lower egg counts (Watson et al., 1986). A reduced faecal consistency may give rise to an increased incidence of fleece dags, predisposing the individual to flystrike and reducing the economic value of the fleece. Previous studies have established that there is a high degree of breed variation in dag score which may be heritable (Meyer, Harvey and Smeaton, 1983; Watson et al., 1986; Baker et al., 1991; Bisset, Vlassoff, Morris, Southey, Baker and Parker, 1992). Watson et al. (1986) found FEC and dag score to be negatively correlated suggesting that selection
for a low incidence of fleece dags may actually select for increased egg count. This result is in agreement with Baker et al. (1991) but opposite to that obtained by Bisset et al. (1992) and McEwan, Mason, Baker, Clarke, Hickey and Turner (1992) who found FEC and the incidence of dags in parasitised Romney sheep to be positively correlated. Recent studies in Australia reported that the incidence of dags did not reflect current worm burdens or nematode egg count (Larsen, Anderson, Vizard, Anderson and Hoste, 1994). Instead the authors suggest that the main cause of diarrhoea and dags in grazing Merinos may be the host’s well-developed inflammatory response, as illustrated by the abundance of mucosal eosinophils, following the ingestion of large numbers of trichostrongylid larvae (Larsen et al., 1994). A similar phenomenon, termed anthelmintic-unresponsive diarrhoea, has been described in grazing Finnish landrace lambs (Suttle and Brebner, 1995). The authors suggest that susceptibility to diarrhoea was linked to an inhibition in nematode egg output and may have been a result of a vigorous mucosal hypersensitivity response to high levels of larval challenge. These results are in accord with recent studies in New Zealand which suggest that dag score may be associated with the level of larval challenge rather than extant worm burden (Douch, Green, Morris and Hickey, 1995a). To date the evidence indicates that more fluid faeces are associated with immunological responsiveness. Thus it appears likely that selection for reduced FEC would lead to an increased frequency of fleece dags.

1.5.5 Specificity of selection

Obviously grazing animals normally come into contact with infective larvae from a number of different nematode species. The potential of selective breeding as a control strategy would be greatly enhanced if increased responsiveness was non-
specific and directed across a range of immunological functions. Evidence from rodent host/parasite models has suggested that selection for responsiveness can be non-specific (Windon, 1990). Biozzi (1982) has demonstrated that mice selected for high circulating antibody levels to foreign red blood cells are more resistant to malaria, trypanosome and helminth infection than those from low antibody lines, but are more susceptible to Salmonella, Brucella, Mycobacterium, Yersinia and Leishmania. Similar findings have been reported from lines of mice selected for responsiveness to single or multiple infection with N. dubius (Brindley and Dobson, 1983b; Sitepu, Dobson and Brindley, 1984). These results suggest that selection for increased responsiveness to one disease organism may select for a particular immunological function to the detriment of others, one consequence of which may be increased susceptibility to other organisms (Dineen, 1985). In support of this hypothesis, data from the Trichostrongylus selection lines suggest that low responder lambs may in fact have a better antibody response to synthetic antigens (Windon and Dineen, 1984).

The evidence available from the Australian selection programmes suggests that sheep selected for improved responsiveness against infection with one species of nematode also express greater resistance to infection with other nematodes (Windon, 1990). Results from the Trichostrongylus selection lines have shown that selection for increased resistance to T. colubriformis may confer some degree of protection towards artificial infection with Trichostrongylus rugatus, T. axei and T. circumcincta (Windon and Dineen, 1984; Windon et al., 1987). Evidence of multi-species, multiple site responsiveness was also apparent following naturally acquired field infection with Trichostrongylus spp. and Teladorsagia spp. Similar results have
been obtained in lambs selected for resistance to *H. contortus*. Lambs from the high responder line exhibited lowered FECs following natural challenge with *Trichostrongylus* spp. and *Teladorsagia* spp. (Woolaston, 1990), and studies in Hungary have shown lambs selected for increased resistance to *H. contortus* to be more responsive to artificial *T. colubriformis* infection (Srèter et al., 1994). Douch (1989) has shown that lambs artificially immunised against *T. colubriformis* displayed increased resistance to natural field infection with *T. axei*, *N. spathiger* and *T. circumcincta*. However, other studies using lambs vaccinated with irradiated larvae or parasite extract of *H. contortus* or *T. colubriformis* showed no evidence of cross-protection following artificial challenge with the other species (Adams, 1989; Adams, Anderson and Windon, 1989). Cross-protection has been shown to involve immunologically specific and non-specific components and it is suggested that for resistance to be expressed against heterologous challenge requires the presence of the homologous "trigger" species (Dineen, Gregg, Windon, Donald and Kelly, 1977).

1.5.6 Selection criteria

In order to best utilise individual variation in host resistance it is important to be able to identify suitable animals for use in breeding programmes. A suitable selection parameter is genetically linked to the resistance trait, does not require prior infection and is unaffected by environmental and physiological influences. Recent research has proposed a number of direct and indirect indicator traits.

The most direct and reliable measure of resistance to nematode infection is an estimation of established worm burden. However as this requires the slaughter of the animal it obviously has no value in breeding programmes. Currently faecal egg count is the simplest and most direct estimate of host responsiveness. Although FEC
provides a good reflection of current $H.\ contortus$ worm burden (Roberts and Swan, 1981) and correlates well with total trichostrongyle burden (McKenna, 1981; Bisset, Vlassoff and West, 1991), it gives a much less accurate estimate for species such as $Teladorsagia$ where current egg count may be less directly related to current worm numbers (Michel, 1969; Jackson and Christie, 1979). However, the use of FEC as an indicator trait is justified as regulation of egg output is one of the key factors in controlling the parasite supra-population (Albers and Gray, 1986).

The relationship between haemoglobin type and resistance to infection was first investigated by Evans, Blunt and Southcott (1963) who found Merinos with haemoglobin type HbAA to be more resistant to artificial $H.\ contortus$ infection than those with HbAB or HbBB blood types. Further studies have confirmed these findings for sheep infected with $H.\ contortus$ (Jilek and Bradley, 1969; Allonby and Urquhart, 1976; Preston and Allonby, 1979b) and $T.\ circumcincta$ (Altaif and Dargie, 1978a). However later studies found no correlation between haemoglobin type and resistance for sheep infected with $H.\ contortus$ (Rifikin and Dobson, 1979; Albers et al., 1984; Luffau, Nguyen, Cullen, Vu Tien Khan, Bouix and Ricordeau, 1986), $T.\ colubriformis$ (Windon and Dineen, 1984) and a mixed $Trichostrongylus$ and $Teladorsagia$ field infection (Riffkin and Yong, 1984).

Australian studies have reported an association between class I lymphocyte antigen allotype, thought to be coded for by genes within or closely linked to the major histocompatibility complex (MHC), and acquired resistance in Merinos to infection with $T.\ colubriformis$ (Outeridge, Windon and Dineen, 1985; Outeridge, Windon, Dineen and Smith, 1986). Two ovine lymphocyte antigens (OLA) of particular interest have been identified. These are the allotypes SY1 and SY2 which
are antigenic variants of sheep class I lymphocyte glycoproteins identified by a microcytotoxicity test (Outteridge et al., 1985). The authors found that SY1 and SY2 were predominant within the high and low responder lines, respectively. In addition, vaccinated animals from the random-bred line expressing SY1 were significantly more resistant than those without. Further experiments confirmed that the association between OLA-type and resistance was not due to sire-related effects (Outteridge, Windon and Dineen, 1988). Studies into the relationship between OLA-type and resistance in Romney sheep in New Zealand also provided similar results (Douch and Outtendge, 1989). However this association has not been confirmed by other studies. No association was discovered between OLA-type and resistance following field infection with Trichostrongylus and Teladorsagia (Riffkin and Yong, 1984) or in sheep selected for resistance to H. contortus (Cooper, van Oorschot, Piper and Le Jambre, 1989). Similarly, Stear et al. (1984) were unable to uncover any relationship between bovine lymphocyte antigens (BoLA) and the resistance of cattle to Cooperia spp. infection. Luffau et al. (1986) did not exclude the possibility of a relationship between OLA-type and resistance to H. contortus and suggested that OLA-type was a potential indicator for resistance in sheep.

Riffkin and Dobson (1979) showed the in vitro responses of ovine lymphocytes exposed to H. contortus antigen prior to infection to be both heritable and positively correlated with subsequent resistance to trickle infection with the parasite. Further studies extended these results to include Trichostrongylus and Ostertagia spp. (Riffkin and Yong, 1984; Riffkin, 1988). However similar results were not found with young lambs from the Trichostrongylus selection lines (Widon and Dineen, 1981). Studies conducted with yearling Saanen goats which had been
raised worm-free failed to find any correlation between the pre-challenge indices and resistance to *H. contortus* infection as measured by worm establishment (Gill, Pomroy, Charleston and Moriarty, 1991).

Recent studies have investigated the relationship between serum antibody levels and the resistance of sheep to nematode infection. Previous studies observed higher anti-*Haemonchus* antibody levels in the serum of genetically resistant Merinos compared to random-bred animals (Gill, Gray, Watson and Husband, 1993b). This is supported by results from New Zealand which suggest that serum antibody levels may reflect overall immunological responsiveness in sheep (Douch et al., 1995a; Douch, Green, Morris, Bisset, Vlassoff, Baker, Watson, Hurford and Wheeler, 1995b). Douch et al. (1995b) have estimated the heritability of variation in serum anti-*T. colubriformis* antibody concentration in 6-months-old Romney lambs to be 0.29 (± 0.08). However, it should be remembered that since antibody levels are influenced both by the innate ability of the sheep to respond and recent exposure to larval challenge, antibody concentration and FEC may not always be in agreement (Douch et al., 1995a).

Modern advances in molecular biology may enable analysis of restriction fragment length polymorphisms (RFLP) to highlight correlations with resistance to nematode infection. Although no detailed results are available at present, Windon (1990) has expressed cautious optimism about their potential use as indicator traits.

A number of other traits have received attention as possible markers for resistance. These include the use of packed cell volume for resistance to *H. contortus* (Albers et al., 1984; Barger and Dash, 1987), frequency of fleece dags, and peripheral eosinophilia which may be associated with the expression of resistance
(Dawkins, Windon and Eagleson, 1989; Buddle, Jowett, Green, Douch and Risdon, 1992). Alternatively animals could be selected for productivity in a parasitised environment (reflecting resilience). However the disadvantage of this approach is that it requires exposure to a moderate degree of infection.

1.5.7 Methods of selection

The type of challenge used to identify responsive individuals may exert considerable influence on the way in which an individual responds. Natural challenge of animals freely grazing on contaminated pasture has considerable merit since it incorporates not only immunoresponsiveness but also physiological and behavioural characteristics which can influence parasite population regulation. However climatic variation between years (particularly in rainfall) may lead to marked differences in levels of pasture larval contamination. Natural infection studies may also be influenced by variation in external stresses such as nutrition and concurrent infection with other pathogens. While artificial challenge allows infection with a controlled number of infective larvae, animals undergoing natural challenge may be exposed to potentially harmful levels of infection or contamination levels too low to elicit a meaningful response. Piper and Barger (1988) provide an example of the problems associated with selection under different levels of infection. The authors concluded that when larval challenge levels were low there was only a small correlation between resistance and production traits but that this was much higher when animals were exposed to higher levels of infection. Most importantly animals selected under one regime should be tested under the other to determine if they show similar levels of responsiveness.
1.5.8 Adaptation of the parasite

One of the major concerns regarding selection for more resistant hosts is the possibility that the parasite, having a considerably higher reproductive rate and much shorter generation interval may be able to adapt to the host immune responses. The obvious example highlighting the parasite’s ability to adapt is the rapid development of anthelmintic resistance. However it is assumed that whereas anthelmintics impose intense selection pressure through a single mode of action, host resistance operating through a variety of mechanisms under polygenic control would not impose a comparable selection pressure (Albers et al., 1984).

The evidence available to date suggests that parasite adaptation to host resistance may not be a problem. After passaging six generations of *H. contortus* through two sheep which had become resistant to infection and nine generations of parasite from the same source through two immunosuppressed sheep, Albers and Burgess (1988) failed to see any significant difference in FEC in susceptible wethers infected with larvae from either strain. The authors argued that the equivalent of three years of selection in the field had produced no observable changes in the *H. contortus* population. Similar conclusions were drawn by Adams (1988) who showed that *H. contortus* was unable to adapt to host acquired immunity after five serial passages. A long term study has been established in Australia to simulate the effect of 15 years of selective breeding for sheep resistant to *H. contortus*. The results after 14 parasite generations in sheep from the *Haemonchus* selection lines suggest that there has been no significant divergence in reproductive fitness between the parasite populations (Woolaston, Elwin and Barger, 1992a). In a laboratory model, no differences were found between lines of *N. dubius* which were passaged for 12
generations in mice selected for resistance to the parasite (Overend, 1985). In contrast studies using the *Trichostrongylus* selection lines have shown that the reproductive fitness of *T. colubriformis* had improved after only one passage through resistant hosts (Windon, 1990).

1.6 Mechanisms of immunity to gastrointestinal nematodes

The majority of our current knowledge regarding the processes involved in the regulation and operation of the immune responses to gastrointestinal helminth infection come from studies conducted using small animals (reviewed by Rothwell, 1989). From these and a number of ruminant studies it is clear that the response to gastrointestinal nematode infection is highly complex and is influenced by age, nutritional and reproductive status, host genotype and the ability of the parasite to evade, suppress or modify the host response (Miller, 1984).

Although immunity in lambs may not fully develop until approximately 6 months of age (Waller and Thomas, 1981; Soulsby, 1985), once acquired, the response to challenge may be very rapid. Challenge of immune sheep with *H. contortus* or *T. colubriformis* showed that the majority of larvae were expelled within 24 hours (McClure, Emery, Wagland and Jones, 1992) and even as quickly as 4 hours post-challenge (Jackson, Miller, Newlands, Wright and Hay, 1988b). Evidence from small animal studies indicates that inflammatory mediators may play a pivotal role in worm expulsion (Rothwell, 1989). Since Stewart (1953) first showed immediate hypersensitivity reactions to be involved in the rejection by sheep of established *H. contortus* adults after *H. contortus* larval challenge, the importance
of local inflammatory reactions in the immune-mediated expulsion of intestinal nematode infections in ruminants has been well documented (Miller, 1984).

The presence of the parasite, the damage caused, worm antigens and other parasite derived factors, all lead to the local recruitment of a variety of lymphoid and myeloid cells. These lymphoid cells include B- and T-lymphocytes, the latter aiding in the regulation of plasma cell, mast cell and eosinophil development and goblet cell hyperplasia. As well as an involvement in the establishment and amplification of the local immune response, T-cell subsets participate in their suppression and provide the memory component of the acquired immune response. Myeloid cells include the eosinophils, neutrophils, basophils, mast cells and monocytes and macrophages.

The mechanisms responsible for elicitation of the acquired immune response have long been thought to be dose-dependent, requiring an antigenic stimulation in excess of some threshold value before an effective response occurs (Dineen, 1963). This has been demonstrated in subsequent studies into the secondary responses of sheep immune to T. circumcincta (Smith, Jackson, Jackson, Williams and Miller, 1984a).

Current information suggests that though worm expulsion may come about through antigen-specific mediators, the resultant inflammatory response may often have non-specific effects on other nematodes in the same or distal parts of the gastrointestinal tract (Stewart, 1953, 1955; Dineen et al., 1977). These mechanisms will be discussed in more detail below.
1.6.1 T-cell response

T-cells have an important influence on the immune response to gastrointestinal nematode infection. Two major subpopulations of T-lymphocytes have been identified in sheep. These are the SBU T4+ and SBU T8+ cells, homologues of human CD4+ (helper) and CD8+ (cytotoxic/suppressor) cells, respectively (Maddox, MacKay and Brandon, 1985). Laboratory studies have shown that protective immune responses elicited following helminth infection are T-cell dependent (Mitchell, 1980). In addition the CD4+ subset plays a major role in protection towards challenge infections with *T. spiralis* and *H. polygyrus* (Grencis, Riedlinger and Wakelin, 1985; Urban, Katona and Finkelman, 1991) and the spontaneous expulsion of *N. brasiliensis* during a primary infection (Katona, Urban and Finkelman, 1988). Recent ovine studies have demonstrated that CD4+ cells play an important role in mediating genetic resistance to *H. contortus* (Gill, Watson and Brandon, 1993a) and increased numbers of these cells have been reported from the lamina propria of *T. colubriformis*-immune sheep (McClure et al., 1992).

Though the precise mechanisms by which CD4+ T-cells regulate ovine resistance to *H. contortus* are unknown, their importance is clear. Depletion of CD4+ T-cells removed differences in susceptibility to *H. contortus* infection between genetically resistant and random bred Merino lambs (Gill et al., 1993a). However, depletion of CD8+ T-cells did not affect host resistance (Gill et al., 1993a). Since there is no evidence that CD4+ T-cells have a direct effect on parasite establishment, fecundity or survival, the authors suggest that they may help regulate the response. This is most likely to be through interactions with parasite antigen and host class II MHC molecules leading to the production of a variety of cytokines which mediate
the recruitment, differentiation and proliferation of immune effector cells such as mast cells, globule leukocytes, eosinophils and antibody-producing cells. The importance of CD4⁺ T-cells in mediating sheep antibody responses towards ovalbumin and *H. contortus* has been reported (Gill, Watson and Brandon, 1992; Gill et al., 1993a). A number of laboratory studies have described the regulatory capabilities of cytokines secreted by CD4⁺ T-cells. Mastocytosis has been shown to be regulated by interleukin-3 (IL-3) and IL-4 (Madden, Urban, Ziltener, Schrader, Finkelman and Katona, 1991), and the induction of eosinophilia by IL-5 (Coffman, Seymour, Hudak, Jackson and Rennick, 1989; Sher, Coffman, Hieny and Cheever, 1990). A similar role for CD4⁺ T-cells was recorded in *H. contortus*-infected lambs (Gill et al., 1993a).

1.6.2 Mast cell and globule leukocyte response

Mastocytosis is a well documented response to helminth infection (Miller, 1984) and is characteristic of nematodiasis in both rodents (Miller and Jarrett, 1971; Ruitenbergen and Elgersma, 1976) and ruminants (Murray, Miller and Jarrett, 1968; O'Sullivan and Donald, 1973). Proliferation in mucosal mast cell (MMC), basophil (Miller and Jarrett, 1971; Askenase, 1980), and in particular globule leukocyte (GL) numbers (Murray et al., 1968; O'Sullivan and Donald, 1973; Huntley, Newlands and Miller, 1984) have been closely associated with the development of resistance to larval establishment and the elimination of adult nematodes, although the precise role of these cells is unclear. In addition, mast cells contain large amounts of the biogenic amine, histamine, which has been implicated as having an effect on both the expulsion of adult worms (Rothwell, 1989) and female worm fecundity (Jones, Windon, Steel and Outteridge, 1990).
The relationship between MMCs and GLs has long been controversial. It has been suggested that MMCs and GLs are totally independent cells (Dobson, 1966; Whur, 1966; Ruitenberg and Elgersma, 1979). However the evidence from laboratory and ruminant studies suggesting that GLs originate from MMCs following antigenic stimulation and degranulation now appears overwhelming (Murray et al., 1968; Miller, 1971; Miller and Walshaw, 1972; Huntley et al., 1984).

There is a great deal of conflicting evidence concerning the importance of MMCs in the immune response. A number of studies have shown a close relationship between mast cell numbers and resistance to nematode infection; *T. colubriformis* in guinea pigs (Rothwell and Dineen, 1972; Handlinger and Rothwell, 1981), *Strongyloides ratti* in rats (Olson and Schiller, 1978), *N. brasiliensis* (Nawa and Miller, 1979) and *T. spiralis* in mice (Alizadeh and Wakelin, 1982a) and *H. contortus* in sheep (Gill et al., 1993a). However other studies have reported mast cell proliferation without worm expulsion (Jarrett, Urquhart and Douthwaite, 1969; Kelly and Ogilvie, 1972) and worm expulsion in the absence of mastocytosis (Ogilvie, Love, Jarra and Brown, 1977; Uber, Roth and Levy, 1980; Dehlawi, Wakelin and Behnke, 1987; Paramentier, de Vries, Ruitenberg and van Loveren, 1987).

Increased GL numbers have been regarded as indicative of resistance to nematode infection in ruminants and laboratory animals (Jarrett, Miller and Murray, 1967; Murray et al., 1968; O’Sullivan and Donald, 1973; Gregg et al., 1978; Huntley et al., 1984; Handlinger and Rothwell, 1981; Douch, Harrison, Elliot, Buchanan and Greer, 1986; Gill et al., 1991; Stankiewicz, Jonas, Douch, Rabel, Bisset and Cabaj, 1993). Although there is a wealth of evidence for an association between MMC and GL numbers and the immune status of the host, studies conducted by Huntley,
Newlands, Jackson and Miller (1992) have suggested that they are not a pre-requisite for immunological regulation of worm burden.

The release of pharmacologically active mediators from MMCs during intestinal nematode infection in laboratory animals (Murray, Miller, Sanford and Jarrett, 1971; Jones, Rothwell, Dineen and Griffiths, 1974) and ruminants (Steel, Jones and Wagland, 1990; Jones et al., 1990; Jones and Emery, 1991) is well documented. These may be released following suitable stimulation via IgE-antigen interactions (Miller, 1984; Rothwell, 1989) and include histamine, serotonin and various chemotactic factors, including proteases, glycosidases and proteoglycans (Durham and Kay, 1985). One particular protease which has attracted considerable interest in ovine studies is sheep mast cell proteinase (SMCP). This protease has chymotrypsin-like esterase activity and has been isolated and purified from sheep MMCs (Huntley, Gibson, Knox and Miller, 1986).

Detection of SMCP levels in blood could be a practical method for monitoring MMC activity in vivo. High concentrations of SMCP have been detected in the blood and gastric lymph of sheep undergoing a protective immune response to *H. contortus* and *T. circumcincta* infections (Huntley, Gibson, Brown, Smith, Jackson and Miller, 1987; Huntley et al., 1992). Similarly, high SMCP levels were detected in the intestinal contents of sheep responding to challenge with *T. colubriformis* (Jones, Huntley and Emery, 1992; Jones, Emery, McClure and Wagland, 1994), suggesting a possible protective role for mast cells.

Other MMC derived factors include the newly formed, membrane-derived mediators. Arachidonic acid may be metabolised by the lipoxygenase pathway to produce leukotrienes (LT) and related compounds, or by the cyclo-oxygenase
pathway to form prostaglandins and associated metabolites (Durham and Kay, 1985). Mast cell products such as prostaglandins and thromboxane are released in *T. colubriformis* infections (Jones and Emery, 1991). Though the consequences of these mediators are unknown they are known to be potent pharmacological agents.

Leukotriene release is well documented in immune rats challenged with *N. brasiliensis* or *T. spiralis* (Moqbel, King, MacDonald, Miller, Cromwell, Shaw and Kay, 1986; Moqbel, Wakelin, MacDonald, King, Grencis and Kay, 1987). Intestinal mediators from sheep resistant to *T. colubriformis* have been shown to contain substances with properties similar to those of slow reacting substance of anaphylaxis (SRS-A), comprising the leukotrienes LTB₄, LTC₄, LTD₄ and LTE₄ (Douch, Harrison, Buchanan and Greer, 1983). In accordance with these results, Jones *et al.* (1990) demonstrated that LTB₄ and LTC₄ secreted into mucus are associated with larval rejection or exclusion.

Despite the evidence for a functional relationship between MMC and parasite expulsion and/or exclusion, one must bear in mind that mast cells are not the only potential sources of pharmacologically active mediators. Thus an absence of MMCs during the immune response should not be taken as conclusive evidence against a possible role for the mediators (Rothwell, 1989).

**1.6.3 Eosinophil response**

The eosinophil has long been associated with worm infection (Jones, 1993) and has been considered an important cellular component of the inflammatory response against helminth parasites (Rothwell and Dineen, 1972). However the precise role of these cells is unclear (Butterworth, 1984) as conflicting results are to be found in the literature. It has been suggested that eosinophilia may be a
consequence rather than a cause of parasite rejection as in several studies involving
*N. brasiliensis* and *S. ratti* infection in rats and *T. colubriformis*-infected guinea pigs
no direct contact between eosinophils and parasite was observed (Rothwell, 1989).
In contrast evidence from a number of host-parasite studies suggests that eosinophils
do play a part in worm expulsion. For example, the ability of strains of mice poorly
responsive to *N. dubius* to expel worms is greatly enhanced after non-specific
eosinophil stimulation (Hurley and Vadas, 1983). Studies with guinea pigs and mice
have reported an association between eosinophilia and worm expulsion (Rothwell
and Dineen, 1972; Dawkins, Carroll and Grove, 1982). However, mice which had
been treated with an antibody against IL-5, negating eosinophil production, showed
no reduction in their ability to eliminate an artificial *Schistosoma mansoni* infection
(Sher *et al.*, 1990).

Though the immunological significance of a pronounced eosinophilia is not
entirely understood, the capacity to mount a rapid and vigorous eosinophil response
may be an important factor in determining the resistance status of the host. This has
been recorded in *T. colubriformis*-infected guinea pigs (Handlinger and Rothwell,
1981) and mice infected with *T. spiralis* (Wakelin and Donachie, 1983) and *H. polygyrus* (Lawrence and Pritchard, 1994). In accordance with these results,
Kimambo, MacRae, Walker, Watt and Coop (1988) reported an association between
high peripheral eosinophil levels and a suppression in *T. colubriformis* FEC in
Suffolk x Finn Dorset wether lambs. These authors suggest that rapid eosinophilia
may be a consequence of the heterotrophic stages of the worm stimulating a host
response (Kimambo *et al.*, 1988).
A number of ovine studies have recorded a similar relationship between blood eosinophil levels and responsiveness (Dawkins et al., 1989; Buddle et al., 1992; Rothwell, Windon, Horsburgh and Anderson, 1993) leading to the suggestion that a marked peripheral eosinophilia may provide an indication of immunological responsiveness rather than reflecting the level of nematode infection. In addition, increased numbers of eosinophils have been recovered from the mucosa of sheep with enhanced resistance to nematodes (Douch et al., 1986) and from lambs genetically resistant to *T. colubriformis* (Dineen and Windon, 1980; Rothwell et al., 1993) and *H. contortus* (Gill, 1991). However other studies have reported a positive association (Dineen et al., 1978), or absence of any observed relationship (Gregg et al., 1978) between intestinal eosinophilia and *T. colubriformis* burden.

Eosinophilia primarily results from a local immune response at the site of infection. Due to their close association with immediate hypersensitivity type reactions, it has been suggested that eosinophils may be attracted by one or more of the mast cell-derived products, such as eosinophil chemotactic factor of anaphylaxis (ECF-A), to sites of mast cell degranulation (Spry, 1988). Though the factors involved in eosinophil regulation are not fully understood, cytokines are known to play an important part (Stevenson and Jones, 1992). IL-5 is now known to be a major regulatory mediator in the development of eosinophilia (Clutterbuck, Hirst and Sanderson, 1989) and eosinophilia associated with helminth infection is abrogated by treatment with monoclonal antibodies towards IL-5 (Sher et al., 1990). In addition eosinophil levels were found to be enhanced in transgenic mice expressing the IL-5 gene (Dent, Strath, Mellor and Sanderson, 1990). Recently, Stevenson and Jones (1992) have described a possible role for cytokines in eosinophil activation through
the development of an enzyme microassay for the detection of eosinophil
potentiating activity (EPA). This enabled detection of EPA in the gastric lymph of
sheep 48 hours after secondary challenge with *T. circumcincta* (Stevenson, Huntley,
Smith and Jones, 1994). Levels of EPA were found to be inversely related to day 10
worm burdens (Stevenson *et al.*, 1994).

The fact that eosinophils appear to be most numerous and in closest contact
with dead parasites has been seen as evidence that they were responsible for parasite
death (MacKenzie, 1980). Studies using laboratory animals have provided evidence
for an effector cell function which results in parasite cell damage (Kazura and Grove,
function is mediated through the degranulation of granule products which are toxic
to the parasite (McLaren, MacKenzie and Ramalho-Pinto, 1977). These toxic
products include oxidative products (Bass and Szejda, 1979; Buys, Wever, van Stigt
and Ruitenberg, 1981), major basic protein (Wassom and Gleich, 1979) and
eosinophil cationic protein (Hamann, Barker, Loegering and Gleich, 1987).
However, not all targets are damaged during these processes (Rothwell, 1989).
Previous studies have demonstrated that IgG (Butterworth, Remold, Houba, David,
Franks, David and Sturrock, 1977), IgE (Capron, Spiegelberg, Bennich, Butterworth,
Pierce, Ouaissi and Capron, 1984) and activated complement (Ramalho-Pinto,
McLaren and Smithers, 1978; Metcalfe, Gadek, Raphael, Frank, Kaplan and Kaliner,
1977) are capable of mediating eosinophil effector function. This function can be
modulated by using a number of soluble cytokines (Silberstein and David, 1987).
The finding that IgA is able to induce eosinophil degranulation (Abu-Ghazaleh,
Fujisawa, Mestecky, Kyle and Gleich, 1989) suggests that IgA and eosinophils may
play an important role in the response to gastrointestinal helminthiasis at mucosal surfaces. Furthermore, IL-5 has been shown to exert its effects on both eosinophils and lymphocytes involved in IgA synthesis (Abu-Ghazaleh et al., 1989). IL-5 may orchestrate the interaction between eosinophils and IgA and thus may play an important role in mucosal immunity.

Genetic variation in the ability to mount an eosinophil response towards parasite infection is well documented (Vadas, 1982; Sewell and Vadas, 1983; Wakelin and Donachie, 1983). As already described previous ovine studies have reported a correlation between eosinophilia and responsiveness (Dawkins et al., 1989). Using Romney lambs Buddle et al. (1992) observed much higher eosinophil levels compared to Merino lambs (Dawkins et al., 1989). It is very possible that apart from breed differences this may also be influenced by differences in age and infection regime (Buddle et al., 1992).

1.6.4 Goblet cell response

Goblet cell hyperplasia is a well documented response to a variety of nematode infections (Rothwell, 1989) and has been recorded from the gastrointestinal tracts of sheep resistant to *O. columbianum* (Dobson, 1967), *H. contortus* (Christie, Hart, Angus, Devoy and Patterson, 1978) and *T. vitrinus* (Jackson et al., 1983). Evidence that goblet cells play a part in the immune response has come from studies which reported a significant increase in goblet cell mucin production correlating with the time of worm expulsion (Miller, Huntley and Dawson, 1981a) with expelled worms trapped in mucus (Miller, 1987b). Further studies found that drugs inhibiting mucus production and function reduced worm expulsion (Miller and Huntley, 1982). A number of studies have suggested that
mucus may help dislodge and trap nematodes, thus aiding in their elimination (Lee and Ogilvie, 1981; Miller, Huntley and Wallace, 1981b; Miller, 1987b; Newlands, Miller and Jackson, 1990). Mucus may also provide a medium for increased retention of antibody and complement thereby enhancing their chance of contact with the worms (Miller, 1987b; Jones et al., 1990).

Mucus may play an important role in rapid expulsion reactions, trapping incoming larvae and preventing their establishment. Indeed, laboratory studies with *T. spiralis* (Lee and Ogilvie, 1981) and *N. brasiliensis* (Miller et al., 1981b) and with sheep infected with *N. battus* (Martin and Lee, 1980) and *H. contortus* (Miller, Jackson, Newlands and Appleyard, 1983a) have shown a close association between mucus and worm expulsion. However, other studies concluded that mucus trapping is not an essential requirement for rapid expulsion (Bell, Adams, and Ogden, 1984).

Mucus has been known to have adverse effects on nematodes for a number of years (Ackert, 1942; Dobson, 1967). Douch et al. (1983) reported the ability of intestinal mucus recovered from sheep immune to *T. colubriformis* to inhibit larval migration in vitro. This larval-paralysis was found to be non-specific and inhibitory activity was ascribed to SRS-A leukotrienes present in the mucus (Douch et al., 1983). Similar paralysing properties of intestinal mucus have been reported by Douch, Harrison, Buchanan and Brunsdon (1984), Douch et al. (1986), Kimambo and MacRae (1988) and Jones et al. (1994).

1.6.5 Immunoglobulin response

The importance of antibodies in ovine acquired immune reactions has been demonstrated by studies where partial resistance to *H. contortus* (Smith, Jackson, Williams, Willadsen and Fehilly, 1984b) and *T. circumcincta* (Smith,
Jackson, Jackson, Graham, Williams, Willadsen and Fehilly, 1986) was transferred from immune to naïve animals using lymphocytes derived from gastric lymph. The transfer of immunity may be due to the triggering of an indirect mechanism by donor cells (Miller, 1984). The results of earlier studies which failed to transfer resistance to *T. colubriformis* to naïve sheep (Adams, 1980) suggest that the antibody response is most effective when combined with other elements of the immune response.

Anti-body responses elicited following ovine gastrointestinal nematode infections are characterised by elevated levels of serum IgG and IgM (Smith, 1977a, b; Charley-Poulain, Luffau and Pery, 1984; Schallig, Van Leeuwen, Bernadina and Hendrikx, 1994) and mucus and lymph IgA (Smith 1977a, b; Adams, Merritt and Cripps, 1980; Smith, Jackson, Jackson, Dawson and Burrells, 1981; Smith, Jackson, Jackson and Williams, 1983b; Smith et al., 1984a,b; Smith et al., 1986; Smith, Jackson, Graham, Jackson and Williams, 1987). These responses may be directed against all stages of the parasite. Recent studies have shown elevated serum IgG1, IgG2 and IgA levels towards L₃ and adult *H. contortus* antigens during primary and secondary infections. Similarly, Charley-Poulain *et al.* (1984) detected raised serum IgG and IgM, and mucosal IgA levels towards adult, L₃ and egg antigens of *H. contortus* in immune sheep.

The local IgA response is possibly the most important and probably the most studied humoral response. Resistance in sheep hyperinfected with *T. circumcincta* has been associated with a secondary response in the gastric lymph consisting of a cellular reaction 2-4 days post-challenge with a marked IgA response a few days later (Smith *et al.*, 1983b, 1984a). Similarly, the association between local and/or systemic IgA and IgG parasite-specific antibody and the resistance of sheep to *H.*
contortus infection is well documented (Smith and Christie, 1978; Charley-Poula"et al., 1984; Gill et al., 1993b; Schallig et al., 1994). However, the importance of IgA in the local immune response has been questioned by studies conducted by Smith, Jackson, Jackson and Williams (1983a) where susceptible lactating ewes showed increased levels of IgA and IgA-containing cells in gastric lymph following challenge with T. circumcincta. The authors suggest that T-cell mediated mechanisms may play a more important role.

Studies with T. circumcincta have proposed that the local antibody response may influence various immune exclusion mechanisms such as parasite stunting, reduced fecundity and worm expulsion (Smith et al., 1985). These effects may be a result of the ability of antibodies to block or neutralise important parasite enzymes (Gill, Husband, Watson and Gray, 1994), or inhibit metabolic processes essential for worm establishment and maintenance (Carlisle, McGregor and Appleton, 1990). In vitro studies with T. colubriformis have shown that worm-specific IgG1 can suppress parasite feeding (Bottjer, Klesius and Bone, 1985).

1.7 Aims

Most of our understanding of immunoresponsiveness and nematode population regulation comes from sheep studies, all of which suggest that the host effector mechanisms which control parasite burdens are complex and that there may be considerable heterogeneity between individuals. Much less is known about nematode population regulation, and the stability and heritability of responsiveness in goats. Enhancing goat herd responsiveness through selective breeding offers the tantalising prospect of reducing reliance upon chemotherapy. The two principal aims
of this thesis are to obtain further information on caprine responses to gastrointestinal nematodes and to examine the potential for selection in temperate climates.
Chapter 2 - Materials and Methods
2.1 Site of study

Field studies were conducted in the Scottish borders at the Sourhope field station of the Macaulay Land Use Research Institute (MLURi), Aberdeen. When required, additional experimental procedures were carried out following housing at the Moredun Research Institute, Edinburgh, where external factors such as nutrition and exposure to nematode infection could be closely controlled. The location of the Sourhope field station is indicated in Figure 1.1. Sourhope is a hill farm, between 300 and 600 metres above sea level, with approximately 1,000 goats and 2,200 ewes which graze improved upland pastures. Although it was not possible to conduct a detailed analysis of the plant composition, the extensive upland pastures also contained some bracken, sedge and small shrubs however the potential to browse exclusively was limited. Mean yearly rainfall measured between 1992-1995 inclusive was approximately 1000 mm, with values of 1057 mm, 1094 mm, 882 mm and 941 mm recorded for the years 1992, 1993, 1994 and 1995, respectively.

2.2 Animals

2.2.1 Goats

All goats used in this study grazed the naturally contaminated hill pastures at the Sourhope research station. Sourhope maintains approximately 1,000 cashmere fibre-producing goats, of which half are breeding does. The goats, termed Scottish cashmere goats, are the product of a selection programme commenced in November 1986 whereby Scottish feral goats have been crossed with imported genetic material from 4 locations, namely Iceland, Tasmania, New Zealand and Southern Siberia.
The Tasmanian and New Zealand stock have the same genetic source and the Icelandic goats are representative of a population of less than 300 individuals.

2.2.2 *Helminth selection-line*

The helminth selection-line was established in the autumn of 1992 with an initial breeding herd of 95 2 to 4-years-old does. These animals were randomly drawn from among the fibre-producing lines maintained at Sourhope and were representative of a wide range of genetic backgrounds.

2.2.3 *Herd management*

In November of each year a proportion of the does were culled due to age, health and suitability for breeding and were replaced with an equal number of yearling females. At the end of the first year of the study these came from the main fibre lines, but in subsequent years replacement animals were drawn from females in the helminth selection line.

An average of one kid per doe was born between late April and early June. Male kids were weaned from their dams at 12 and females at 16 weeks of age. Males and females from the helminth selection-line were grazed in single sex groups alongside an equivalent number of control animals prior to housing over their first winter. Housed kids were accommodated in straw bedded paddocks and kept on a diet of equal parts hay and ‘Green Keil’ (Central Farmers Ltd., Fife, Scotland, 16% crude protein). Yearling animals were turned out onto pasture in early May. Breeding bucks were selected in October/November of each year.
Sourhope practices an annual drug rotation regime, alternating between ivermectin (Oramec, MSD Agvet, at 200 μg kg⁻¹ body weight) and levamisole (Nilverm SD, Pitman-Moore Ltd., at 7.5 mg kg⁻¹ body weight), with goats normally treated every 5 weeks while on pasture. In the first season (1992-93) the does were treated with levamisole.

2.2.4 Conventional sheep

Conventionally reared sheep, where used, were Scottish Blackface ewes aged between 2 and 4 years that had grazed naturally contaminated pastures at one of the Institute farms.

2.2.5 Worm naïve lambs

Seven-month-old Scottish Blackface lambs which had been reared under worm-free conditions since birth were used to provide primary challenge data.

2.3 Parasitological techniques

2.3.1 Infective larvae

Infective larvae for artificial challenge were cultured from faeces collected from monospecifically infected donor lambs. Faeces were incubated in plastic trays at 22°C for 10 days, soaked in warm tap water (22°C) for 1 hour and then the fluid decanted and allowed to sediment for 2 hours at 4°C. After sedimentation the volume was reduced and the larvae cleaned by Baermanisation through high wet-strength paper (Cleanaroll Ltd.). Larval yield was estimated by calculating the
number of larvae present in an aliquot of the larval suspension. Infective larvae were stored in tap water at 4°C and used within 3 weeks of being harvested.

2.3.2 Artificial challenge regimes

A number of artificial challenge regimes were used over the course of these studies. These regimes have commonly been used in ovine challenge studies conducted at Moredun (F. Jackson, personal communication). Where the response to artificial and natural infection was to be investigated, housed animals were given an artificial challenge of 10,000 *T. circumcincta* and/or 10,000 *T. vitrinus* L₃ three weeks before being turned out onto contaminated pasture. In order to control for external factors such as differences in nutrition and the level of exposure to natural infection, selected goats were housed at Moredun and given an artificial trickle challenge consisting of 2000 *T. circumcincta* and 1000 *T. vitrinus* L₃ per day, 5 days per week for 4 weeks. The dosage used in these continuous challenge models (CCM) was designed to mimic that to which the goats would be exposed while on naturally infected pastures (F. Jackson, personal communication). As *T. circumcincta* is the most important gastrointestinal nematode parasite of sheep and goats in Scotland (Urquhart et al., 1987) the ability of selected goats to regulate the establishment and development of this parasite was an important part of this study. This ability of the host was determined by killing 10 days after a single artificial challenge with 50,000 *T. circumcincta* L₃ at which time the established larvae would be expected to be developing to the fifth/adult-stage (Denham, 1969). In order to reduce the numbers of experimental animals required, this single challenge was given to housed goats which had been treated with anthelmintic after completing a period of artificial
trickle infection. This had the added benefit of ensuring that all animals which were
given the single artificial challenge had had similar levels of recent exposure to the
parasite. Similar anthelmintic disrupted challenge models (ADCM) have been used
in previous challenge studies conducted at Moredun with *T. circumcincta* (Smith *et

2.3.3 Faecal egg counts

Faecal egg counts were performed using a modified flotation technique as
described by Christie and Jackson (1982). Rectal faecal samples were taken into a
300 x 250 mm, 100 gauge polythene bag and assigned a score reflecting their
consistency. This faecal score ranged from 1 for a sample consisting almost wholly
of blood and mucus to a score of 5 for a sample consisting of dry, hard pellets. The
sample was then weighed and 10 ml of tap water added per gramme of faeces prior
to emulsification. A 10 ml sub-sample was then removed and passed over a 1 mm
aperture sieve. The retentate was washed through with an additional 5 ml of tap
water, compressed to recover as much fluid as possible and then discarded. The
filtrate was poured into a 20 ml polyallomer centrifuge tube (Beckman, USA) and
centrifuged at 1000 rpm (228 g) for 2 minutes. The supernatant was removed and the
faecal pellet resuspended gently in 12 ml saturated sodium chloride solution prior to
recentrifugation (1000 rpm for 2 minutes). Using artery forceps, the tube was
clamped just below the meniscus and the contents of the upper chamber washed into
a 4 ml disposable polystyrene cuvette (LIP Ltd., Shipley). The cuvette was filled with
saturated salt solution and sealed. Counts were conducted on a compound
microscope using a calibrated eyepiece graticule (Miller square) at ×40
magnification. The eyepiece graticule enabled the total number of eggs in concentrated samples to be calculated after counting a subsample of the eggs present. Two longitudinal traverses of the cuvette were performed, and the numbers of eggs falling inside the boundaries of the large graticule square multiplied by 3 or those within the small square by 9 to obtain the total number of eggs per gramme of faeces. In those samples with very low egg concentrations all the eggs were counted.

2.3.4 Specific faecal egg counts

The proportion of each nematode species present in pooled faecal samples was determined as described by Christie and Jackson (1982). Eggs were extracted and counted as described above. The individual samples within each group were pooled, concentrated over a 38 μm aperture sieve and added to saturated salt solution in a cuvette as described for egg counts. The eggs were viewed at ×60 magnification using a strain gauge image-shearing module (Vickers M14/2) fitted to a Vickers M14-compound microscope. In this way the lengths and breadths of 50 eggs were recorded and the dimensions of each egg used to determine the relative species abundance.

2.3.5 Post-mortem procedures

All animals were either stunned with a captive-bolt pistol and exsanguinated, or given a lethal barbiturate overdose (Nembutal, SANOFI Animal Health Ltd.). The animal was opened along the ventral midline and ligatures placed at the omasal/abomasal, abomasal/duodenal and ileo/caecal junctions prior to the removal of the organs.
The entire gastrointestinal tract was removed and the proximal end of the small intestine located. The proximal two-thirds of the small intestine were separated from the mesentery and the contents collected. Sections of the small intestine (4-8 cm long) were taken approximately 2 m from the proximal end and fixed for histopathology. The small intestine was then opened longitudinally and together with its contents soaked in 0.85% saline solution at 37°C for 4 hours. Following this saline digest the intestine was drawn gently between finger and thumb to remove the superficial mucosa and then discarded. The intestinal contents were made up to 5 litres with 0.85% saline, and a 500 ml (10%) subsample taken and fixed with 20 ml formalin.

The abomasum was opened along its greater curvature and the contents collected. An entire abomasal fold was removed and fixed for histopathology. Following saline digest as described above, the abomasum was discarded and the abomasal contents made up to a volume of 5 litres. A 10% subsample (500 ml) was taken and fixed with 20 ml formalin.

2.3.6 Worm counts

A 100 ml aliquot, representing 2% of the total worm population, was taken from each of the fixed subsamples and stained with 10-15 ml of helminthological iodine (Appendix A). The stained sample was washed over a 38 μm aperture sieve to remove excess iodine before being examined under a stereo microscope. Any worms present were recovered and preserved in 2% formalin. Potentially anomalous high or low worm counts were repeated. *T. circumcincta* larvae were staged as early, mid or
late L₄ or fifth-stage/early adult as described by Denham (1969). *T. vitrinus* larvae were staged according to Douvres (1957).

2.4 Haematology

2.4.1 Collection of blood samples

Blood samples were taken at the same time of day for each time point within an experiment to reduce the effect of diurnal variation. Samples were collected by jugular venepuncture into 10 ml ethylenediaminetetracetate (EDTA) containing vacutainer tubes (Becton-Dickinson Ltd., Oxford, UK). In the lymph cannulation experiments blood was obtained via an indwelling jugular catheter. After conducting total white cell counts and preparing samples for enumeration of peripheral eosinophil levels (as described below), the remaining blood was centrifuged (2500 rpm, 1430 g, for 15 minutes) and the plasma removed and stored at -20°C.

2.4.2 White cell counts

Total white cell counts were conducted using a model ZM Coulter Counter (Coulter Electronics Ltd., Luton, UK) calibrated to detect particle size down to 3.617 μm diameter. White cell numbers are expressed as \( \times 10^9 l^{-1} \).

2.4.3 Peripheral eosinophil counts

Two methods were used to conduct peripheral eosinophil counts over the course of this study. In the first experiment the red blood cells were lysed and the percentage eosinophil numbers determined from differential counts made of 600 leukocytes on cell smears fixed and stained with Diff-Quik (Brownes Ltd., Reading,
UK) as per the manufacturer’s instructions. The smears were prepared in a
cytocentrifuge (Shandon Scientific Ltd., Runcorn, UK) using 0.2 ml of white cell
suspension adjusted to contain $10^6$ cells ml\(^{-1}\) and spun at 600 rpm (50 g) for 5
minutes. These cytospot smears were air dried for 5 minutes before staining.
Absolute eosinophil numbers were then estimated by relating the percentage count to
the total white cell count.

In all subsequent experiments peripheral eosinophil numbers were calculated
using a second method. A 50 µl sample of fresh blood was added to 450 µl of
Carpentier's eosinophil staining solution (Appendix A) and the eosinophils counted
using a “Fast-Read 10” disposable counting chamber (Immune Systems Ltd., Bristol,
UK). Blood eosinophil numbers are expressed as $\times10^9$ l\(^{-1}\).

2.5 Histology

2.5.1 Tissue preparation

Sections of the abomasum and small intestine were removed immediately
after slaughter and fixed in 4% weight/volume paraformaldehyde in phosphate
buffered saline (PBS) pH 7.4 (Appendix A) for 6 hours (Newlands, Huntley and
Miller, 1984). Tissues were dehydrated through a graded ethanol series, cleared in
toluene and embedded in paraffin wax (Miller et al., 1983a). Tissue sections were
cut to 5 µm using a microtome (Leica Instruments GmbH, Milton Keynes, UK),
dewaxed in xylene and rehydrated prior to staining.

2.5.2 Mast cell and globule leukocyte counts
For histochemical enumeration of mast cells, tissue sections were stained overnight with toluidine blue at pH 0.5 (Enerback, 1966). For immunohistochemical localisation of SMCP, showing both mast cells and globule leukocytes, sections were incubated with affinity-purified rabbit antibody to SMCP followed by polyvalent sheep F(ab')₂ anti-rabbit Fab-horseradish peroxidase as described by Huntley et al. (1986). Differential globule leukocyte counts were made after staining with carbol chromotrope (Appendix A) for 30 minutes (Lendrum, 1944).

Stained sections were washed thoroughly in tap water, dehydrated in ethanol, cleared, air dried and permanently mounted with Coverbond mounting medium (American Hospital Supply Corporation, Illinois, USA). Stained cells were counted under a compound microscope with a ×10 eyepiece containing a calibrated graticule and a ×40 objective lens giving a viewing area of 0.08 mm². For abomasal sections, the counts were made from the muscularis to the mucosal surface on a minimum of 20 fields from 3 separate sections of the fold, and are expressed as the number of cells 0.2 mm⁻² abomasal mucosa. Small intestinal counts were made and expressed per villus crypt unit (VCU).

2.5.3 Tissue eosinophil counts

Tissue eosinophil counts were conducted on carbol chromotrope stained sections. Eosinophils were counted as described for globule leukocytes, and the results expressed as the number of eosinophils 0.2 mm⁻² of mucosa or per VCU for the abomasum and small intestine, respectively.
2.6 Lymphatic cannulation

2.6.1 Surgical procedures

The animals were starved for 24 hours prior to being anaesthetised with a halothane-nitrous oxide-oxygen mixture. Anaesthesia was induced by face mask and maintained through an endotracheal tube via a closed circuit apparatus.

The common gastric lymph duct (Nickel, Schummer and Seiferle, 1976) was cannulated as described elsewhere (Smith et al., 1981). The peritoneal cavity was opened just behind the last rib on the right side. The wound was held open using a Cleland retractor set and the liver retracted cranially. The abomasum was exposed and a few hundred µl of 1% Evans blue in saline were injected into it subserosally. Blunt dissection between the ventral wall of the dorsally retracted vena cava and the dorsal aspect of the pancreas revealed the blue-stained lymph vessel emerging cranially from behind the portal vein and running caudally and dorsally over the pancreas. The lymph duct was tied off and catheterised according to Lascelles and Morris (1961) with a polyvinyl chloride cannula (Duraplastics and Engineering, Sydney, Australia) of 1.20 mm external diameter. The cannula was led out through a small incision high and far back on the flank and the individual muscle layers and
Plate 2.1. Lymph duct cannulation showing lymph ducts stained with Evan’s blue.

2.6.2 Collection and reinfusion of gastric lymph

The procedures used in these studies are a modification of those described previously by Smith et al. (1983a). Each animal was fitted with a harness to which was attached a 2 l disposable urine-drainage bag (Type MP4, Sterilin Ltd., Teddington, UK) fitted with a non-return valve and drainage port, and containing 20 ml of holding solution (Appendix A). Every morning each bag was weighed, a 10 ml sample of overnight lymph withdrawn and the remaining contents reinfused via the jugular catheter. During reinfusion, 5 mls of fresh lymph were collected and a new bag attached. Lymph flow, measured as the difference in bag weight over the 24 hour period, was recorded daily.
Teddington, UK) fitted with a non-return valve and drainage port, and containing 20 ml of holding solution (Appendix A). Every morning each bag was weighed, a 10 ml sample of overnight lymph withdrawn and the remaining contents reinfused via the jugular catheter. During reinfusion, 5 mls of fresh lymph were collected and a new bag attached. Lymph flow, measured as the difference in bag weight over the 24 hour period, was recorded daily.

2.6.3 Lymph composition

The total white cell counts of fresh and 24 hour lymph samples were determined daily using a model ZM Coulter Counter (Coulter Electronics Ltd., Luton, UK), calibrated to detect particle size down to 3.617 μm diameter. Cell smears were prepared from 0.2 ml of fresh lymph adjusted to a lymphocyte concentration of $2 \times 10^6$ ml$^{-1}$ as previously described.

2.6.4 Enumeration of blast and IgA-containing cells

Lymphoblast cells in efferent gastric lymph were identified from cytospots stained with Leishman's stain (Appendix A). The slides were flooded with neat Leishman's stain for 5 minutes followed by 10 minutes in stain diluted 1:1 with tap water. Slides were then washed with tap water and allowed to air dry. IgA-presenting cells were identified after prior immunoglobulin staining as described below. Cells were counted using a compound microscope with a ×10 eyepiece containing a calibrated graticule and a ×40 objective lens. The response was determined from differential counts performed on a minimum of 500 lymphocytes in 3 different fields of view, and absolute cell numbers calculated by relating this value to the total white cell count obtained previously.
2.7 Immunological and biochemical techniques

2.7.1 Enzyme-linked immunosorbent assay for sheep mast cell proteinase

Sheep mast cell proteinase concentrations in tissue homogenate were determined using a double antibody sandwich enzyme-linked immunosorbent assay (ELISA) as described by Huntley et al. (1987).

Excised tissue samples were washed in PBS, weighed and stored at -20°C. The tissues were minced finely and suspended in 10 ml of emulsifying buffer (Appendix A) prior to homogenisation using a laboratory Mixer Emulsifier (Silverson Machines). The plates were incubated overnight at 4°C with rabbit anti-SMCP in carbonate/bicarbonate buffer pH 9.6 (Appendix A) (1 μg ml⁻¹, 50 μl per well). Tissue samples were diluted 1:10, 1:100 and 1:1000 in wash buffer (0.5% Tween 20 in PBS) and run in duplicate. The SMCP standards were run in duplicate at concentrations of 0.5, 1, 2, 4, 6, 8, 10 and 12 ng ml⁻¹ in wash buffer. Control samples were incubated in uncoated wells. After washing with wash buffer, samples and standards were added to the wells (50 μl per well) and incubated at room temperature for 1 hour. Following washing, 50 μl horseradish peroxidase-conjugated rabbit anti-SMCP antibody (diluted 1:2000) was added to each well and the plate incubated for 1 hour at room temperature. After further washing the bound antibody was reacted with freshly prepared orthophenylenediamine (OPD) in H₂O₂ phosphate-citrate buffer pH 5.0 (150 μl per well) (Appendix A) for 5 minutes. The reaction was stopped with 25 μl of 2.5 M sulphuric acid and the optical density (OD) at 492 nm determined on a Titertek multiscan ELISA reader (Titertek Flow Laboratories). SMCP concentrations are expressed as μg SMCP g⁻¹ wet weight of tissue.
2.7.2 Enzyme-linked immunosorbent assay for chicken serum albumin

Optimal conditions were determined by checkerboard titration. Best results were obtained using a serum dilution of 1:1000 and a conjugate dilution of 1:800.

Flat-bottomed microtitre plates (Dynatech Laboratories Ltd., Billinghamurst, Sussex, UK) were coated overnight at 4°C with chicken albumin (100 µl per well) diluted 1 mg ml$^{-1}$ in 0.1 M carbonate-bicarbonate buffer pH 9.6 (Appendix A), and subsequently washed with wash buffer (Appendix A). Test serum (50 µl per well) diluted 1:1000 in serum/conjugate diluent (Appendix A) was run in triplicate, the plates incubated for 1 hour at 4°C and washed with wash buffer. Horseradish peroxidase-conjugated donkey anti-goat IgG (Serotec) diluted 1:800 in serum/conjugate diluent was added to each well (50 µl per well) and the plates incubated for a further 1 hour at 4°C. Following incubation, the plates were washed with wash buffer and distilled water. Orthophenylenediamine (Sigma Fast OPD dihydrochloride tablet set) was prepared as per the manufacturer's instructions and added (200 µl per well) as the enzyme substrate. The reaction was stopped after 30 minutes in the dark at room temperature with the addition of 3M sulphuric acid (50 µl per well) and the OD measured at 492 nm.

2.7.3 Immunoglobulin staining of lymphocytes

Lymph smears were prepared by cytocentrifugation as previously described. Once dried, slides were fixed in 2% paraformaldehyde in PBS for 20 minutes and then washed for 10 minutes in Tris buffer pH 7.5 (Appendix A). The smears were flooded with mouse anti-sheep IgA (Sigma) diluted 1:25 in 0.05% TWEEN 20 in PBS. Slides were placed in a humid box at room temperature for 1½ hours and
washed in PBS prior to addition of horseradish peroxidase-conjugated goat anti-
mouse IgG (Sigma) diluted 1:50 in normal goat serum. The slides were again placed
in a humid box for 1½ hours at room temperature, washed in PBS, flooded with
carbozole substrate (Appendix A) for 5 minutes and finally rinsed thoroughly with
tap water.

2.8 Statistical analysis

Statistical analysis was performed using Minitab version 9.2 statistical
software. Arithmetic means together with the standard error of the mean (± SEM)
are given unless stated otherwise. Egg counts, peripheral eosinophilia and post-
mortem data were analysed using the untransformed mean value for each individual,
and the effect of the group was examined using the Mann-Whitney test. Faecal egg
count repeatability estimates were obtained by calculating the degree of correlation
between adjacent log_{10}(x + 1) transformed samples within each group. Correlation
estimates ranged from -1.00 to +1.00, with a value of 0.00 indicating an absence of
any association and a value of 1.00 indicating a linear relationship: In the absence of
statistical analysis a correlation value of at least 0.75 was required before a
relationship was deemed to be important. Estimates of repeatability of FEC were
analysed using the 1-sample Wilcoxon test. Data transformation and the statistical
tests used are described in each chapter.

Estimates of heritability of log_{e}(x+ 1) transformed FEC were calculated by
Residual Maximum Likelihood fitting an Animal Model using computing techniques
as described by Meyer (1989). The heritability values, which range from 0.00 to
1.00, describe the proportion of variation between animals that is due to differences
in genetics. Egg count data while on pasture were acquired for the bucks in 1992, does in 1992-1993, male and female yearlings from both the selected and control lines in 1994 and male selected and control line yearlings in 1995. Heritability estimates calculated using mean FECs provide the most accurate measure of heritability as they measure an individual animal over a number of sampling periods. The heritability value obtained for a single FEC is less accurate as only one count is performed for each individual.
Chapter 3 - Comparison of responses of female sheep and goats to gastrointestinal nematode infection
3.1 Introduction

Previous studies conducted in Australia using 15-month old Angora and Merino wethers grazing pasture infected predominately with *H. contortus*, *T. circumcincta* and *T. colubriformis* have demonstrated that goats are considerably more susceptible to gastrointestinal nematode infection than are sheep (Le Jambre and Royal, 1976; Le Jambre, 1984a). These results have been supported by studies with naturally infected grazing adult Romney sheep and feral goats in New Zealand (Pomroy et al., 1986). However, if goats are able to browse plant material higher than 12.5 cm above ground level they may be exposed to lower levels of challenge and exhibit lower worm egg counts than do grazing sheep (Silangwa and Todd, 1964; Vercruysse, 1983). Breed and parasite differences have a major influence on host resistance to infection. Red Masai sheep in Kenya have been shown to be considerably more resilient to *H. contortus* infection than 3 other sheep and 3 goat breeds (Preston and Allonby, 1979a), while Le Jambre and Royal (1976) showed that Angora wethers harboured significantly more of all species of gastrointestinal nematodes except *Nematodirus* than did Merinos of a comparable age.

A wealth of evidence has been compiled from sheep and rodent studies suggesting an important role for the host’s cellular response following gastrointestinal nematode infection (see review by Miller, 1984). The immunoregulatory response to infection is highly complex, involving mucosal mastocytosis, globule leukocyte and goblet cell hyperplasia and peripheral and tissue eosinophilia (Rothwell, 1989). Previous studies have shown the inflammatory response, and in particular the presence of globule leukocytes, to be closely associated with gastrointestinal helminthiasis in sheep (O’Sullivan and Donald,
1973; Huntley et al., 1984; Huntley et al., 1992) although the precise role of these cells is as yet unclear. Similar findings from laboratory studies (Alizadeh and Wakelin, 1982b; Miller, Woodbury, Huntley and Newlands, 1983b) suggest that the type-I immediate hypersensitivity reactions are an important element in the immune-mediated expulsion of gastrointestinal helminths (Miller, 1984).

Although the relative susceptibility of goats to nematode infection is well documented, it is unclear as to whether there are underlying differences in the mechanisms by which sheep and goats respond to nematode infection. The aim of this study was to obtain preliminary data on the differences in immunologically mediated effector mechanisms employed by sheep and goats in the regulation of their gastrointestinal nematode populations. The sheep used were Scottish Blackface ewes, a hill breed which previous studies have suggested to be more resilient to nematode infection (Abbott et al., 1985).

3.2 Materials and Methods

3.2.1 Animals

Eight Scottish Blackface ewes and 8 feral-cross does aged between 2 and 4 years-old were used. Both ewes and does were rearing twin offspring at the start of the study. Five 7-month-old Blackface lambs which had been born and reared indoors under worm-free conditions were used to provide parasitological and immunological data on primary challenge of naïve lambs. Comparable worm-naïve goat kids were not available.
3.2.2 Grazing study

The does and ewes were housed and treated with ivermectin (Ivomec, MSD Agvet at 200 μg kg\(^{-1}\) bodyweight) before being turned out onto the same contaminated pasture in early May. Every five weeks thereafter, a rectal faecal sample was obtained from each animal prior to drenching (ivermectin, 200 μg kg\(^{-1}\)).

3.2.3 Faecal egg counts

Faecal egg counts were performed as described in Chapter 2.

3.2.4 Challenge study

An outline of the experimental protocol used in this study is shown in Table 3.1. In mid-October the ewes and does were treated with levamisole (Levacide, Norbrook Animal Health, 12 mg kg\(^{-1}\)) and fenbendazole (Panacur, Hoechst Ltd., 10 mg kg\(^{-1}\)) and housed together with 5 worm-free lambs (day 0 of challenge study). On day 15 all animals were orally challenged with a mixed infection of 10,000 *T. circumcincta* and 10,000 *T. vitrinus* third-stage larvae. Eleven days post-challenge (day 26) all animals were killed using a captive-bolt pistol.

Table 3.1. Outline of experimental timetable used for artificial challenge of ewes, does and naive lambs.

<table>
<thead>
<tr>
<th>Day of Challenge Study</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Housing; administration of levamisole and fenbendazole.</td>
</tr>
<tr>
<td>0</td>
<td>Conduct blood eosinophil counts.</td>
</tr>
<tr>
<td>13</td>
<td>Conduct blood eosinophil counts.</td>
</tr>
<tr>
<td>15</td>
<td>Artificial challenge infection.</td>
</tr>
<tr>
<td>20</td>
<td>Conduct blood eosinophil counts.</td>
</tr>
<tr>
<td>26</td>
<td>Conduct blood eosinophil counts.</td>
</tr>
<tr>
<td>26</td>
<td>Kill; conduct worm counts and post-mortem analyses.</td>
</tr>
</tbody>
</table>
3.2.5 Eosinophil counts

Venous blood was collected on days 0, 13, 20 and 26 post-challenge as described previously. Total white cell numbers were calculated using an improved Neubauer haemocytometer after staining with Tuerk’s white cell diluting fluid (Appendix A). Percentage and absolute eosinophil numbers were calculated as outlined in Chapter 2.

3.2.6 Post-mortem techniques

The abomasum and small intestine of each animal were removed and processed as described in Chapter 2. The numbers, sex and stage of development of the worms recovered from 2% aliquots were determined.

Immediately after slaughter excised sections of abomasal and jejunal folds were processed for detection of mast cells, globule leukocytes, tissue eosinophils and sheep mast cell proteinase concentrations as described earlier (Chapter 2).

3.2.7 Statistical analysis

Faecal egg counts and worm burden data were analysed on Minitab using one-way analysis of variance on log_{10}(x + 1) transformed data. Post-mortem tissue values were analysed using log_{10}(x + 1) transformed data and differences between the group means determined by the Student’s two-sample t-test. The rates of decline in peripheral eosinophilia were compared in ewes and does using a paired t-test.
3.3 Results

3.3.1 Faecal egg counts

The mean (± SEM) faecal egg counts of the ewes and does at pasture are shown in Figure 3.1. At each sampling point between August and October does had significantly higher egg counts than did ewes (p<0.001).

![Figure 3.1. Mean (± SEM) FECs of ewes and does maintained on pasture between May and October.](image)

3.3.2 Worm burdens

The total abomasal worm burdens and the numbers present as fourth stage larvae are shown in Figure 3.2. Table 3.2 contains details of the mean worm populations, including the stages of larval development and the percentage of the established larvae recovered at the fourth larval stage. Mean *T. circumcincta* burdens (± SEM) were 3688 (± 649) in the does, 763 (± 223) in ewes and 2690 (± 451) in the lambs. Ewes had significantly lower numbers of *T. circumcincta* than lambs (p<0.05) and does (p<0.005).
Of the established larvae, significantly more were present as L₄ in ewes (85%) compared to does (71%, p<0.05) and lambs (44%, p<0.001). The proportion of worms recovered which were still at the L₄ stage was significantly higher in does than lambs (p<0.001). It is clear from the individual worm burdens that there was considerable variation in the resistance of the does to infection; the highest *T. circumcincta* burden (6150) being eight times larger than the smallest (750).

The total numbers of *T. vitrinus* recovered from each individual are shown in Figure 3.3. Mean (± SEM) doe worm numbers were 3630 (± 448) compared with 2730 (± 567) in naïve lambs and only 19 (± 13) in the ewes (Table 3.3). Ewes harboured significantly fewer *T. vitrinus* than either the lambs or does (p<0.001). All of the worms recovered from the ewes were L₄ compared to 86% in does and 78% in lambs. These values were not significantly different. There was a high degree of variation in doe *T. vitrinus* burdens, the most susceptible individual harbouring over twice as many worms (5350) as the most resistant (2250). This variation was similar to that seen in the *T. circumcincta* populations.
**Figure 3.2.** Individual *T. circumcincta* burdens recovered from does, ewes and lambs post-challenge.

**Figure 3.3.** Individual *T. vitrinus* burdens recovered from does, ewes and lambs post-challenge.
### Table 3.2. Mean (± SEM) *T. circumcincta* burdens.

<table>
<thead>
<tr>
<th>Group</th>
<th>Early L₄</th>
<th>Mid L₄</th>
<th>Late L₄</th>
<th>Stage 5</th>
<th>Total</th>
<th>% Established larvae as L₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Does</td>
<td>1059 (± 295)</td>
<td>676 (± 143)</td>
<td>884 (± 191)</td>
<td>1069 (± 260)</td>
<td>3688 (± 649)</td>
<td>71²</td>
</tr>
<tr>
<td>Ewes</td>
<td>218 (± 93)</td>
<td>301 (± 97)</td>
<td>131 (± 76)</td>
<td>113 (± 46)</td>
<td>763 (± 223)</td>
<td>85²</td>
</tr>
<tr>
<td>Lambs</td>
<td>0 (± 0)</td>
<td>20 (± 20)</td>
<td>1160 (± 227)</td>
<td>1510 (± 267)</td>
<td>2690 (± 451)</td>
<td>44³</td>
</tr>
</tbody>
</table>

Figures with different superscripts are significantly different (see text).

### Table 3.3. Mean (± SEM) *T. vitrinus* burdens.

<table>
<thead>
<tr>
<th>Group</th>
<th>XL₃</th>
<th>Early L₄</th>
<th>Mid L₄</th>
<th>Late L₄</th>
<th>Stage 5</th>
<th>Total</th>
<th>% Established larvae as L₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doe</td>
<td>26 (± 26)</td>
<td>550 (± 550)</td>
<td>298 (± 112)</td>
<td>2268 (± 481)</td>
<td>488 (± 138)</td>
<td>3630 (± 448)</td>
<td>86</td>
</tr>
<tr>
<td>Ewes</td>
<td>0 (± 13)</td>
<td>19 (± 13)</td>
<td>0 (± 0)</td>
<td>0 (± 0)</td>
<td>0 (± 0)</td>
<td>19 (± 13)</td>
<td>100</td>
</tr>
<tr>
<td>Lambs</td>
<td>0 (± 119)</td>
<td>0 (± 217)</td>
<td>220 (± 313)</td>
<td>1900 (± 567)</td>
<td>610 (± 7)</td>
<td>2730 (± 567)</td>
<td>78</td>
</tr>
</tbody>
</table>

Figures with different superscripts are significantly different (see text).
3.3.3 Eosinophil counts

Circulating eosinophil levels in the ewes declined significantly (p<0.05) after housing and anthelmintic treatment on day 0 (Figure 3.4). There were no significant changes in eosinophil numbers in the does or ewes post-challenge.

![Graph showing eosinophil counts](image)

**Figure 3.4.** Peripheral eosinophil counts of ewes and does after housing.

There were no significant differences between the three groups in tissue eosinophil numbers from abomasal (Table 3.4) or small intestinal sections (Table 3.5). Abomasal tissue eosinophil numbers were weakly positively associated with the proportions of L4 recovered from the ewes and does (r = 0.52 and r = 0.68, respectively). This relationship was not detected in the naïve lambs.

3.3.4 Mast cell responses

Mast cells, as stained with toluidine-blue, were significantly more numerous (p<0.001) in sections of abomasum (Table 3.4) and small intestine (Table 3.5) taken from ewes as compared with sections from does or lambs (Plates 3.1 and 3.2).
Table 3.4. Mean (± SEM) abomasal mast cell numbers calculated after staining with toluidine blue and anti-SMCP. Also shown are globule leukocyte (GL), proportion of mast cells as GLs (%GL) and tissue eosinophil (Eos) numbers.

<table>
<thead>
<tr>
<th>Group</th>
<th>Toluidine Blue</th>
<th>Anti-SMCP</th>
<th>GL</th>
<th>% GL</th>
<th>Eos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Does</td>
<td>3.6a (± 0.7)</td>
<td>22a (± 4)</td>
<td>15.7a</td>
<td>81.3</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(± 4.3)</td>
<td>(± 4.3)</td>
<td>(± 0.4)</td>
</tr>
<tr>
<td>Ewes</td>
<td>30b (± 5.1)</td>
<td>32.3a (± 5.2)</td>
<td>8a</td>
<td>21.1</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(± 2.6)</td>
<td>(± 4.4)</td>
<td>(± 0.2)</td>
</tr>
<tr>
<td>Lambs</td>
<td>2.6a (± 0.9)</td>
<td>1.0b (± 0.4)</td>
<td>0b</td>
<td>0.0</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(± 1.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figures with different superscripts are significantly different (see text).

Immunohistochemical staining, revealing both MMCs and GLs, showed that lamb abomasal tissue had significantly fewer SMCP-containing cells than did that of ewes and does (p<0.005). Significantly higher numbers of SMCP-containing cells were recovered from ewe small intestine than from lambs (p<0.01). There were no significant differences in SMCP-containing cell numbers in doe and ewe tissues.

That those SMCP-containing cells not revealed with toluidine-blue were GLs was confirmed after staining with carbol chromotrope. No GLs were present in worm-free lamb tissue. Does had significantly more abomasal and intestinal GLs than lambs (p<0.005) and significantly more intestinal GLs than did ewes (p<0.001, Plates 3.3 and 3.4). Ewes had significantly more jejunal (p<0.05) and abomasal (p<0.005) GLs than lambs. Significantly higher numbers of GLs were recovered from...
doe small intestine than from ewes (p<0.005) though there was no difference in abomasal values.

Abomasal worm burden was negatively associated with the abundance of SMCP-containing cells and GLs in does (r = -0.76 and r = -0.68, respectively) and ewes (r = -0.52 and r = -0.74, respectively). No similar relationship was detected for the small intestine.

Table 3.5. Mean (± SEM) intestinal mast cell numbers calculated after staining with toluidine blue and anti-SMCP. Also shown are globule leukocyte (GL), proportion of mast cells as GLs (%GL) and tissue eosinophil (Eos) numbers.

<table>
<thead>
<tr>
<th>Group</th>
<th>Toluidine Blue</th>
<th>Anti-SMCP</th>
<th>GL</th>
<th>% GL</th>
<th>Eos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Does</td>
<td>10.7a (± 2.3)</td>
<td>29.6 ±3.7</td>
<td>22.9a (± 3.4)</td>
<td>68.2 ±4.0</td>
<td>13.8 (± 1.3)</td>
</tr>
<tr>
<td>Ewes</td>
<td>62.9b (± 13.3)</td>
<td>63.9a (± 12.5)</td>
<td>4.6b (± 2.2)</td>
<td>6.8 (± 1.8)</td>
<td>9.7 (± 0.8)</td>
</tr>
<tr>
<td>Lambs</td>
<td>10.8a (± 2.9)</td>
<td>15.6b (± 4.9)</td>
<td>0.0a (± 0.6)</td>
<td>0.0 (± 0.6)</td>
<td>10.7</td>
</tr>
</tbody>
</table>

Figures with different superscripts are significantly different (see text).

3.3.5 SMCP concentrations

Mean tissue SMCP concentrations measured in the abomasum and small intestine (Table 3.6) of ewes were significantly greater (p<0.001) than those found in the corresponding tissues of does or lambs. SMCP concentrations were similar in the
does and lambs. There was no consistent relationships between SMCP concentrations and worm burdens or larval development.

Table 3.6. SMCP concentrations (µg g⁻¹ w/w) obtained from tissue homogenate.

<table>
<thead>
<tr>
<th>Group</th>
<th>Abomasum</th>
<th>Small Intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Does</td>
<td>0.20ᵃ</td>
<td>3.2ᵃ</td>
</tr>
<tr>
<td></td>
<td>(± 0.07)</td>
<td>(± 0.8)</td>
</tr>
<tr>
<td>Ewes</td>
<td>92.4ᵇ</td>
<td>71.4ᵇ</td>
</tr>
<tr>
<td></td>
<td>(± 23.9)</td>
<td>(± 22.6)</td>
</tr>
<tr>
<td>Lambs</td>
<td>0.8ᵃ</td>
<td>2.1ᵃ</td>
</tr>
<tr>
<td></td>
<td>(± 0.4)</td>
<td>(± 0.6)</td>
</tr>
</tbody>
</table>

Figures with different superscripts are significantly different (p<0.001).
Plate 3.1. Photomicrograph illustrating the frequency of mucosal mast cells in the sheep small intestine.

Plate 3.2. Photomicrograph illustrating the frequency of mucosal mast cells in the goat small intestine.
Plate 3.3. Photomicrograph illustrating the frequency of globule leukocytes in the sheep abomasum.

Plate 3.4. Photomicrograph illustrating the frequency of globule leukocytes in the goat abomasum.
3.4 Discussion

These results clearly demonstrate the susceptibility of Scottish cashmere goats to gastrointestinal nematode infection and are similar to previous studies comparing Merino and Angora wethers (Le Jambre, 1984a) and adult feral goats and Romney sheep (Pomroy et al., 1986). They also provide initial evidence that there are fundamental differences between sheep and goats in their immunological responses to nematode infection.

Does shed significantly more eggs, particularly during the latter part of the grazing season, than did ewes. Over the period of field study mean doe egg count was almost 3 times that of the Blackface ewes. Although FEC may not accurately reflect the true level of infection, particularly for genera such as Teladorsagia where egg output tends to follow a stereotypic pattern (Michel, 1969; Jackson and Christie, 1979), analysis of individual worm burdens post-challenge supported the observation that ewes were significantly more refractory to infection.

Previous studies investigating the development of resistance to *T. vitrinus* and *T. circumcincta* proposed that one of the first manifestations of acquired immunity in young lambs was a reduction in the rate of development of incoming larvae (Seaton et al., 1989a, b). Host resistance to infection may be expressed also through a reduction in the numbers of eggs shed per female worm. This reduction in worm fecundity has been described in studies on the development of immunity in lambs to *T. colubriformis* and *H. contortus* infection (Dineen and Windon, 1980; Gill et al., 1991). The results of the present challenge study suggest that acquired immunity to nematode infection in ewes was manifested in two ways: firstly, a reduction in larval establishment, and secondly, a retardation in the rate of larval
development. Not only did ewes harbour extremely low post-challenge worm burdens, but the vast majority of the worms present had progressed no further than the fourth larval stage. In contrast, does, although they had had extensive prior exposure to nematode infection, had considerably larger abomasal and intestinal burdens than did worm-naïve lambs. Nevertheless, though there was little apparent effect on worm numbers, there was evidence of immunological and/or physiological arrest of larval development, with a significantly higher proportion of individual *T. circumcincta* burdens present as L₄ in does when compared to lambs. These results, and in particular the wide variation in worm numbers, support the view that adult goats may acquire and express some degree of immunity to gastrointestinal nematode infection although clearly this facility is not as well developed as that in Blackface ewes. However, as worm-naïve goat kids were not available, it was not possible to investigate this.

A peripheral and tissue eosinophilia is characteristic of helminthiasis (Jones, 1993) and may play an important role in host resistance to nematode infection (Rothwell and Dineen, 1972; Dawkins et al., 1989). The results of the present study suggest that continuous antigenic stimulation is required to maintain circulating eosinophil numbers as blood eosinophil levels declined significantly after housing and anthelmintic treatment. At slaughter, the challenge control lambs had higher abomasal tissue eosinophil levels than either does or ewes though all groups had similar mean intestinal numbers. Previous sheep and rodent studies have attempted to quantify the role of tissue eosinophils as effector cells in response to nematodiasis. However the conclusions are unclear as a positive relationship between tissue eosinophils and worm burden has been reported in one study (Dineen et al., 1978),
no correlation by another (Gregg et al., 1978) and a weak negative correlation in a study of selected responder and non-responder lambs (Dineen and Windon, 1980). Other studies have reported that eosinophil levels may correlate with the host’s ability to mount an immune mediated response (Handlenger and Rothwell, 1981; Dawkins et al., 1989; Rothwell et al., 1993). The fact that ewes and does in this study had very similar tissue eosinophil numbers suggests that mucosal eosinophils are unlikely to play a direct role in the response to nematode infection although an indirect role cannot be ruled out. With respect to the higher tissue eosinophil numbers recovered from the challenge control lambs, previous studies with *H. contortus* infection in randomly bred lambs have shown there to be considerable differences in mucosal eosinophil levels, with higher numbers found after primary than secondary challenge (Gill, 1991).

Mucosal mastocytosis and globule leukocyte and goblet cell hyperplasia are well documented features of the host response to gastrointestinal nematode infection (Miller, 1984). In the current study abomasal and jejunal mast cells were significantly more numerous in ewes compared to does and lambs. Total mast cell numbers in ewe abomasal and small intestinal tissues tended to be inversely related to total worm numbers. There was a similar inverse relationship between doe abomasal mast cell numbers and total *T. circumcincta* burden, though this was not the case for the small intestine. However the overriding tendency in lamb abomasal and jejunal sections was for higher total mast cell numbers in those individuals with high worm populations. There was no evidence of any consistent relationship between mast cell numbers and larval development. Mastocytosis has been recorded following intestinal protozoan infection (Huntley, Newlands, Miller, McLauchlan,
Rose and Hesketh, 1985) and it is possible that the high intestinal MMC counts obtained in this study may have been confounded by the effects of exposure to other intestinal pathogens. There is conflicting evidence as to the importance of MMCs in the immune response, some studies showing tissue mast cells to be correlated with resistance (Handlinger and Rothwell, 1981; Gill et al., 1993a), other studies reporting the accumulation of mast cells without subsequent worm expulsion (Kelly and Ogilvie, 1972) and worm expulsion in mice in the absence of mastocytosis (Uber et al., 1980; Paramentier et al., 1987). The data collected from this study tend to indicate that mast cells may play an important role in the immune response following nematode infection, their increased presence in ewe and doe abomasal tissue indicative of individuals which were mounting a more effective response. The linear relationship between mast cell numbers and total worm burdens in lambs and the absence of any beneficial effect on slowing larval development rates may be explained when the previous exposure of the animals is considered. Both does and ewes had had considerable experience of gastrointestinal nematode infection and would thus be expected to mount an anamnestic response. In contrast to this, the lambs had been exposed to infection for a relatively short period (11 days) and were mounting a primary immune response. Previous single challenge serial kill studies (Armour et al., 1966) with Teladorsagia have shown relatively stable worm populations until after day 14 post-challenge. It appears that in the challenge control lambs mast cell recruitment had occurred but effector function clearly had not. These immature mast cells can also be difficult to detect in stained sections (Rothwell, 1989).
Globule leukocytes are degranulated mast cells (Huntley et al., 1984) whose abundance in gut mucosa has been shown to be closely correlated with resistance to infection (O'Sullivan and Donald, 1973; Gregg et al., 1978; Handlinger and Rothwell, 1981; Gill et al., 1991). However, these observations conflict with those of the present study. Although does had many more globule leukocytes than did ewes they appeared largely unable to regulate their worm burdens. Indeed, it is interesting to observe that adult does and worm-naive lambs possessed similar numbers of mast cells, that no globule leukocytes were detected in lamb tissue but that mean doe worm burdens were very much higher. If the worm expulsion mechanisms are similar in the two species these results suggest that the mucosal response in goats is much less effective than that of sheep (even those with no previous experience of nematode infection) at regulating worm establishment. This is in concordance with the findings of Huntley et al. (1992) who suggested that the presence of large numbers of mast cells or globule leukocytes was not a pre-requisite for immunological regulation of gastrointestinal nematode populations.

Previous studies have associated increased levels of mucosal SMCP with host rejection of gastrointestinal infection (Huntley et al., 1987; Huntley et al., 1992; Jones et al., 1994). In the current study ewes had significantly higher SMCP levels in both abomasal and jejunal tissues than did either does or lambs. Doe and lamb tissue SMCP concentrations were very similar though does had more SMCP-containing cells. Maximal SMCP levels in worm-naive sheep have been shown to occur at least 7-8 weeks after primary infection (Dobson et al., 1990; Bendixsen, Emery and Jones, 1995). In contrast, significant SMCP releases have been recorded from immune sheep 6-8 days after challenge with T. colubriformis (Bendixsen et al., 1995).
Exposure to infection may sensitise mucosal mast cells to larval antigens resulting in a greater release of SMCP and, concomitantly, an increase in the numbers of globule leukocytes post-challenge (Bendixsen et al., 1995). This contrasts with the findings of the present study where, although does had very low SMCP concentrations, they had considerably higher numbers of globule leukocytes. These findings suggest that goat mast cells may contain considerably less of the ‘SMCP-like’ enzyme than sheep mast cells. However it is possible that there are differences in the release of SMCP between sheep and goats and also that the peak SMCP release may well have occurred prior to the time of slaughter in this study and so would not have been detected. It is also important to take into consideration that the ELISA used for the detection of SMCP in this study was designed for use with sheep mast cells, and that even though there would appear to be a close antigenic homology between SMCP and an assumed goat mast cell proteinase (J. F. Huntley, personal communication), this ELISA may not be able to reliably measure the concentrations of the caprine proteinase accurately. More definitive studies on the composition of goat mast cell proteinase are obviously required.

The results from this study outline clear differences in the immune effector mechanisms of adult sheep and goats in response to gastrointestinal nematode infection. Ewes were able to regulate both the establishment and development rates of established worms considerably more effectively than the does or worm-naïve lambs. This was coupled with significantly elevated numbers of toluidine-blue stained mast cells and significantly higher SMCP concentrations though there were no significant differences in total mast cell numbers between ewes and does. In contrast, globule leukocytes were much more prevalent in doe than ewe tissue. That
the does harboured substantially higher worm burdens than the ewes and even the
worm-naïve lambs suggests that they have a considerably less efficient mechanism
for the immune exclusion of the parasites. However, the considerable variation in
doe worm populations and rates of larval development suggest that individual goats
may be able to express a degree of acquired immunity to gastrointestinal nematode
infection.

These results illustrate clear differences between sheep and goats in their
relative susceptibilities to and immunological responses following gastrointestinal
nematode infection. Although the does were more susceptible to infection than were
ewes and worm naïve lambs, there was evidence of a high degree of individual
variation in responsiveness. The more resistant does had lower worm populations
with a higher proportion of retarded larvae and possessed greater numbers of tissue
globule leukocytes.
Chapter 4 - Responses of entire male Scottish cashmere goats to gastrointestinal nematode infection: segregation into responders and non-responders
4.1 Introduction

Gastrointestinal nematode parasitism has a significant detrimental effect on animal production throughout the world (Holmes, 1985). As most current control strategies are heavily reliant on chemotherapy the increasing prevalence of anthelmintic resistance, particularly among the nematode parasites of small ruminants, is of major concern to producers world-wide (Jackson, 1993). This problem is particularly acute in goats with widespread anthelmintic resistance reported throughout the world (Varady, Praslicka and Corba, 1994). Recent studies have isolated a strain of *Teladorsagia* from fibre-producing goats in Scotland which exhibits a degree of multiple resistance towards both ivermectin and the benzimidazoles (Jackson *et al.*, 1992a).

Previous studies have demonstrated that goats are considerably more susceptible to gastrointestinal nematode infection than sheep (Le Jambre and Royal, 1976; Le Jambre, 1984a; Pomroy *et al.*, 1986). Moreover, differences in drug pharmacokinetics and the degree of rumen bypass may lead to reduced drug bioavailability and subsequently less efficacious drench treatments in goats (Sangster *et al.*, 1991). The resultant underdosing is an important factor in the rapid development of anthelmintic resistance (Martin, 1990). Though the goat industry is still very small in Scotland (with approximately 10,500 goats), the fact that sheep and goats are often grazed together on upland pastures where they become infected with the same nematode species, together with the potential of goats to seed pasture with large numbers of nematode eggs (Anon, 1982), mean that goats may pose a significant threat to the economically more important sheep industry.
The prevalence of anthelmintic resistance in the UK and the speed at which it can develop (Jackson, 1993) has been one stimulus for increased research into sustainable control strategies. One of the most encouraging options is to utilise the high degree of between- (Stewart et al., 1937), and within-breed (Albers et al., 1984) variation in the immunological responsiveness of sheep to gastrointestinal nematode infection. Selective breeding programmes in Australia and New Zealand have successfully produced lines of Merino and Romney sheep with increased resistance to *H. contortus, T. colubriformis* and *Teladorsagia* spp. infection (Windon, 1991).

Considerably less research has been directed towards identifying differences in resistance to infection in goats. Studies with *H. contortus* infection in Kenya (Preston and Allonby, 1978) and gastrointestinal strongyles in France (Richard et al., 1990) have demonstrated that goats do exhibit genetic variation in responsiveness. In contrast, the authors of a recent caprine breeding study in Fiji reported very little genetic variation or repeatability in FEC following natural mixed *T. colubriformis/H. contortus* and monospecific *T. colubriformis* infection (Woolaston et al., 1992b).

However it is unclear whether the absence of variation in responsiveness was due to a true lack of variation of expression in goats or reflected differences in management, nutrition and exposure to infection in the tropics.

Few studies have focused upon the susceptibility of post-pubertal male ruminants to gastrointestinal nematode infection (Barger, 1993a); most work having been conducted using female or wether lambs. To date, evidence available from the *Trichostrongylus* and *Haemonchus* selection programmes in Australia suggests that entire male lambs are more susceptible to infection than females from the same lines (Windon, 1991). However the reasons for this increased susceptibility are unclear.
The studies described here investigated the individual responsiveness of entire adult male Scottish cashmere bucks exposed to both natural infection at pasture and artificial infection when housed. The aims of the study were to identify those animals occupying the outlying positions of responsiveness using simple parasitological parameters. The most resistant individuals would then be used as the first sires in a selective breeding programme for increased resistance to nematode infection.

4.2 Materials and Methods

4.2.1 Animals

One hundred 2-4 years-old intact male Scottish cashmere goats were gathered together at the Sourhope Research station in the Scottish borders. Forty of these animals originated from the Sourhope farm, the remainder were drawn from 11 other cashmere-producing herds throughout Scotland. Seventeen bucks were rejected on the grounds of their unsuitability for use in a future breeding programme, leaving a final study group of 83 animals. The Scottish cashmere bucks used in this study were representative of a number of diverse genetic backgrounds, deriving from native animals and genetic material imported from New Zealand, Tasmania, Iceland and Southern Siberia as described in Chapter 2. All bucks had had previous exposure to the nematode species used in these studies.

4.2.2 Identification of responder and non-responder bucks

In early May 1992, all 83 bucks were housed to prevent exposure to uncontrolled parasite infection and treated with anthelmintic (ivermectin, Oramec, at 400 μg kg\(^{-1}\) body weight). Three weeks later they were given a single artificial
challenge of 10,000 *T. circumcincta* L₃ per os, and three weeks post-challenge turned out to pasture. Previous studies have shown these pastures to be contaminated predominately with *T. circumcincta* and *T. vitrinus* larvae (Jackson *et al.*, 1992b).

Individual faecal egg counts were performed weekly after turnout. In early August, 7 weeks post-turnout, and 10 weeks after artificial infection, all bucks were treated with fenbendazole (Panacur, 5 mg kg⁻¹). Faecal egg count data collected from mid-August onwards thus derive either from drug resistant worms that had survived treatment or from natural reinfection.

Resistance to gastrointestinal nematode infection was assessed on the basis of egg count over a 15 week period on pasture. Egg counts were ranked each week, the lowest count being assigned a ranking of 1, the highest 83. At the end of the period of field study (late September), a cumulative ranking was obtained from the previous weekly rankings. The 6 bucks with the lowest cumulative rankings were deemed to be most resistant to infection and were designated as responders, the 6 with the highest rankings were deemed to be least responsive and termed non-responders.

4.2.3 Faecal egg counts

Faecal samples were scored and egg counts performed as described previously (Chapter 2). Specific faecal egg counts were conducted at time of slaughter using the methods described in Chapter 2.

4.2.4 Breeding programme

At the end of the field study, the top 2 responder bucks (F8002 and C9053) were used to impregnate 95 does in the first year of the selective breeding programme. Following housing and trickle challenge infection the 3 best responders
(F8002, D1195 and H8088) were kept aside for use in the second year's breeding programme.

4.2.5 Artificial challenge of selected responder and non-responder bucks

The timetable used for the housing and artificial trickle challenge of the selected responder and non-responder bucks in 1993 and 1994 is shown in Table 4.1.


<table>
<thead>
<tr>
<th>Time Period</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>May-October 1992</td>
<td>Identification of responders and non-responders from 83 bucks following artificial/natural infection on pasture.</td>
</tr>
<tr>
<td>October 1992</td>
<td>Top responders F8002 and C9053 used in first year of breeding programme.</td>
</tr>
<tr>
<td>February 1993</td>
<td>6 responder and 5 non-responder bucks housed at Moredun.</td>
</tr>
<tr>
<td>July 1993</td>
<td>Commenced trickle challenge 21 days after last ivermectin treatment.</td>
</tr>
<tr>
<td>October 1993</td>
<td>Killed non-breeding bucks. Top responders F8002, D1195 and H8088 used in second year's breeding programme.</td>
</tr>
<tr>
<td>January 1994</td>
<td>3 responders F8002, D1195 and H8088 housed, given 10 consecutive treatments with ivermectin.</td>
</tr>
<tr>
<td>February 1994</td>
<td>21 days after last ivermectin treatment the 3 bucks underwent an identical trickle challenge regime as in 1993.</td>
</tr>
<tr>
<td>March 1994</td>
<td>F8002, D1195 and H8088 were killed.</td>
</tr>
</tbody>
</table>

In February 1993 the selected responder and non-responder bucks were housed at Moredun to control for external factors such as differences in nutrition and level of infection and treated with fenbendazole (Panacur, 10 mg kg⁻¹). Unfortunately one of the non-responders had died prior to housing from causes other than
parasitism leaving a challenge study group of 6 responder and 5 non-responder bucks. The animals were housed in one group on a straw covered concrete floor under conditions designed to minimise the risk of accidental infection. Over the period of housing the animals were maintained on a diet of ESCA nuts (East of Scotland College of Agriculture, 14% crude protein) and hay and water ad libitum.

Faecal egg counts conducted 3 weeks after administration of fenbendazole showed this treatment to have been largely unsuccessful. In an attempt to remove the remaining worms the bucks were treated with ivermectin (Oramec, 200 µg kg⁻¹) at the beginning of March. Three weeks later the goats were given a combination of levamisole (Levacide, 12 mg kg⁻¹) and fenbendazole (Panacur, 10 mg kg⁻¹) followed the next day with ivermectin (Oramec, 200 µg kg⁻¹). One month later, towards the end of April, a drug pharmacokinetics study was conducted using fenbendazole (Panacur, 5 mg kg⁻¹) the results of which will be reported elsewhere. Egg counts were finally reduced to zero following treatment with ivermectin at twice the recommended dose rate (400 µg kg⁻¹) on 10 consecutive days.

Artificial trickle infection commenced 21 days after the last anthelmintic treatment (day 0 of the challenge study). This consisted of a mixed oral challenge of 2000 T. circumcincta and 1000 T. vitrminus L₃ per day, 5 days per week for 4 weeks. Both laboratory parasite strains were susceptible to the three broad spectrum anthelmintic families. Faecal egg counts were conducted twice weekly following first infection.

On day 60 after first infection the non-breeding bucks were killed. The 3 top responders (F8002, D1195 and H8088) were selected for use in the second year’s breeding programme and so were not killed with the other bucks. In order to obtain
post-mortem data from these breeding responders the artificial challenge regime was repeated with these animals the following year. The 3 bucks were re-housed in January 1994, treated with anthelmintic (Oramec, 400 μg kg⁻¹ for 10 consecutive days), and challenged as before, beginning in early February. On day 60 the bucks were killed and post-mortem analysis conducted as described below.

4.2.6 Eosinophil counts

Venous blood samples were taken weekly following first infection. Peripheral eosinophil numbers were determined after fixing in Carpentier’s stain as described in Chapter 2. Total white cell counts were conducted using a model ZM Coulter Counter (Coulter Electronics Ltd., Luton, UK).

4.2.7 Post-mortem techniques

The abomasum and small intestine of each animal were removed, and the numbers, sex and stage of development of the worms recovered from 2% aliquots determined. Excised sections of abomasal and jejunal folds were processed for the enumeration of mast cells, globule leukocytes and tissue eosinophils as described in Chapter 2.

4.2.8 Statistical analyses

The arithmetic mean (± SEM) is given in all instances. Statistical analyses were conducted on the untransformed mean data for each animal and the effect of the group tested using the Mann-Whitney test. Repeatability estimates for egg count were obtained by calculating the degree of correlation between adjacent log₁₀(x + 1) transformed samples within each group. Analysis of estimates of FEC repeatability were conducted using the 1-sample Wilcoxon test.
4.3 Results

4.3.1 Identification of responder and non-responder bucks

The mean (± SEM) egg counts of the selected 6 responder and 6 non-responder bucks while on pasture are shown in Figure 4.1. Also included for comparison are the mean (± SEM) counts for the entire herd of 83 animals. Retrospective analysis of FECs up to the time of anthelmintic treatment in early August showed the selected responders to have a significantly lower mean FEC resulting from artificial and natural challenge than non-responders (70 ± 22 epg and 252 ± 63 epg, respectively, p<0.005). The herd mean (154 ± 11 epg) was significantly different from that of both responders and non-responders (p<0.001). Post-treatment FEC from natural reinfection was significantly lower in responders (58 ± 11 epg) than both non-responders (241 ± 25 epg, p<0.001) and the herd mean (133 ± 13 epg, p<0.005). Analysis of mean egg count over the entire period of artificial and natural challenge showed responders to have shed significantly fewer eggs than had non-responders (64 ± 7 and 247 ± 18 epg, respectively, p<0.005). Responder and non-responder counts were significantly different from the herd mean (144 ± 12 epg, p<0.001).

Although there was a degree of variability in the rankings obtained for individual bucks over the period on pasture, selected responders and non-responders were consistently ranked within the top or bottom 10 animals, respectively. Mean estimates of repeatability of adjacent log transformed egg counts on pasture were statistically significant for both responders (0.25 ± 0.10, p<0.05) and the entire herd (0.31 ± 0.05, p<0.001). The mean estimate obtained for non-responders was not significantly different from zero. Repeatability estimates calculated on the mean
**Figure 4.1.** Mean (± SEM) FECs for the selected 6 responder and 6 non-responder bucks together with the herd mean following artificial and natural challenge.

**Figure 4.2.** Mean (± SEM) faecal scores of samples obtained from bucks following artificial and natural challenge.
herd FECs were significant both before and after anthelmintic treatment (0.33 ± 0.09, p<0.05 and 0.29 ± 0.05, p<0.05, respectively). Pre- and post-treatment estimates for responders and non-responders were not significantly better than zero.

Scoring of individual faecal samples showed a significant reduction in faecal consistency in all groups 3 weeks after turnout (responders and non-responders p<0.05, herd mean p<0.001). This was most pronounced in the selected responders and corresponds to the time of rapidly increasing egg counts from pasture infection (Figure 4.2).

4.3.2 Housing of responders and non-responders

The mean faecal egg counts of the 6 responder and 5 non-responder bucks after housing are shown in Figure 4.3. During the course of the infection (day 24) two bucks, one responder and one non-responder, sustained injuries as a result of fighting and were removed from the study. Since neither of these animals had completed the period of challenge, the data from them have been disregarded. There were no significant differences between mean responder and non-responder FECs at housing. Mean responder egg count in the 3 weeks following initial fenbendazole treatment was significantly reduced (20 ± 5 epg, p<0.01) and was significantly less than that of non-responders (157 ± 23 epg, p<0.05). Although non-responder egg counts were still high, they were significantly lower than pre-treatment (p<0.05). Responder egg counts remained low for the duration of housing though egg production was not completely halted. During the 6 week period following fenbendazole/levamisole and ivermectin treatment, mean non-responder FEC was significantly greater than that of responder bucks (451 ± 84 and 24 ± 5 epg, respectively, p<0.01).
Figure 4.3. Mean (± SEM) FECs of selected 6 responder and 5 non-responder bucks post-housing and prior to challenge infection.

Figure 4.4. Mean (± SEM) faecal scores of selected 6 responder and 5 non-responder bucks post-housing and prior to challenge infection.
The administration of fenbendazole as part of the pharmacodynamics study had no observable effect on egg production. Mean responder counts (36 ± 3 epg) remained significantly lower than those of non-responders (515 ± 38 epg, p<0.01) until all animals were successfully treated. Over the period of housing, during which time the bucks were kept free from re-infection, mean responder egg counts were only one-tenth those of non-responders (43 ± 7 and 423 ± 59 epg, respectively, p<0.01).

There were no within-group correlations between mean FEC at pasture and after housing. Grouping the responders and non-responders together into a single group gave an estimate of repeatability between mean FEC on pasture and after housing of 0.90. Over the period of housing mean responder body weight was slightly greater than that of non-responders, though this difference at no time approached significance (data not shown).

Non-significant differences were apparent in faecal consistency score between responders and non-responders (Figure 4.4). Mean responder faecal score over the period of housing prior to artificial infection was slightly lower than that of non-responders (3.89 ± 0.02 and 3.97 ± 0.01, respectively).

4.3.3 Artificial challenge infection

The arithmetic mean (± SEM) FECs for the 5 responder and 4 non-responder bucks which completed the first challenge study in 1993 are shown in Figure 4.5. Mean responder egg count between days 21 and 60 after first infection was significantly lower than that of non-responders (187 ± 36 and 553 ± 48 epg, p<0.05). Estimates of FEC repeatability were statistically significant in both responder and non-responder animals (0.62 ± 0.13, p<0.01 and 0.73 ± 0.09, respectively, p<0.005).
There was no evidence of any correlation between mean pasture or housed and post-challenge egg counts in either group. However taking the egg counts as a single group showed a high degree of repeatability between mean FEC on pasture and after housing with post-challenge egg counts in 1993 (0.72 and 0.81, respectively).

Figure 4.6 compares of the mean FECs of the 3 breeding responders following identical challenge in 1993 and 1994. Although there were no significant differences at each point after infection, mean egg count was significantly lower in 1994 than 1993 (134 ± 15 and 72 ± 14 epg, respectively, p<0.005). Mean estimate of repeatability of FEC in 1994 was 0.54 (± 0.18), statistically significant (p<0.05) and similar to that obtained the previous year (0.55 ± 0.19).

In order to obtain a more meaningful picture of the responses of selected bucks to artificial challenge infection, and to enable comparison with post-challenge worm burdens, the faecal egg count data for the 3 responders challenged in 1994 were added to that collected for the other bucks in 1993. Though there were differences in FEC of the 3 responders between 1993 and 1994, the egg count response followed a similar pattern over both years and was very different to that of the non-responders. The overall mean egg counts of the selected responder and non-responder bucks are displayed in Figure 4.7.
Figure 4.5. Mean (± SEM) FECs of selected 5 responder and 4 non-responder bucks following trickle challenge, 1993.

Figure 4.6. Mean (± SEM) FECs of the 3 breeding responder bucks following trickle challenge in 1993 and 1994.
Mean responder egg count between days 21-60 after first infection was significantly lower than that of non-responders (145 ± 34 and 565 ± 48 epg, respectively, p<0.05). In addition, responders had significantly lower counts between days 24-38 and 49-60 (p<0.05). Estimates of repeatability of FEC were statistically significant in both responders and non-responders (0.77 ± 0.06 and 0.72 ± 0.11, respectively, p<0.001). Combining responder and non-responder egg counts into a single group showed mean FECs on pasture and after housing to be strongly correlated with post-challenge egg counts (0.74 and 0.80, respectively).

![Graph](image)

**Figure 4.7.** Mean (± SEM) FECs of selected 5 responder and 4 non-responder bucks following trickle challenge in 1993 and 1994.

### 4.3.4 Post-challenge worm burdens

Total abomasal worm burdens showing the numbers of worms recovered at the L4 and fifth/adult stages are displayed in Figure 4.8. Mean *T. circumcincta* burdens, including the stages of larval development recovered at day 60 are shown in Table 4.2. Table 4.3 shows the proportion of the established larvae recovered at each
stage of larval development. Responders harboured on average 4970 (± 1600) T. 
circumcincta compared to a mean non-responder burden of 13,390 (± 3420).

Although non-responders had almost 3 times as many worms this difference was not
statistically significant. Of the established worms, a higher proportion were present
as L₄ in responders (14.0%) than non-responders (3.8%). Fifth/adult stage male to
female ratios were similar in responders and non-responders (1:1.25 and 1:1.07,
respectively). The degree of correlation between fifth/adult stage female T.
circumcincta numbers and specific egg counts taken at time of slaughter were high
in both responder and non-responder bucks (r = 0.78 and r = 0.85, respectively).
Mean fecundity (eggs per female per gramme faeces) was only slightly lower in
responders than non-responders (0.062 ± 0.011 and 0.065 ± 0.013, respectively).

Individual T. vitrinus burdens showing the numbers of fourth and fifth/adult
stage worms recovered from responders and non-responders are shown in Figure 4.9.
Mean T. vitrinus burdens, stages of larval development and numbers of male and
female fifth/adult stage worms are shown in Table 4.4. Responders harboured a
mean of 3680 (± 1299) worms, significantly fewer than the non-responder mean of
8875 (± 1240, p<0.05). Table 4.5 records the proportion of established larvae
recovered at each stage of development.
### Table 4.2. Mean (± SEM) *T. circumcincta* worm burdens.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Early L₄</th>
<th>Mid L₄</th>
<th>Late L₄</th>
<th>Stage 5/Adult Male</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responders</td>
<td>53 (± 53)</td>
<td>292 (± 187)</td>
<td>350 (± 236)</td>
<td>1895 (± 780)</td>
<td>2380 (± 578)</td>
</tr>
<tr>
<td>Non-responders</td>
<td>273 (± 84)</td>
<td>122 (± 41)</td>
<td>123 (± 41)</td>
<td>6212 (± 1688)</td>
<td>6660 (± 1730)</td>
</tr>
</tbody>
</table>

### Table 4.3. Mean (± SEM) percentage composition of abomasal worm burdens.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Early L₄</th>
<th>Mid L₄</th>
<th>Late L₄</th>
<th>Stage 5/Adult Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responders</td>
<td>1.1 (± 0.5)</td>
<td>5.9 (± 3.0)</td>
<td>7.0 (± 2.4)</td>
<td>38.1 (± 3.7)</td>
<td>47.9 (± 6.8)</td>
</tr>
<tr>
<td>Non-responders</td>
<td>2.0 (± 0.7)</td>
<td>0.9 (± 0.4)</td>
<td>0.9 (± 0.4)</td>
<td>46.4 (± 5.6)</td>
<td>49.8 (± 6.3)</td>
</tr>
</tbody>
</table>

Non-significant differences were apparent in the rates of larval development though a higher proportion of the established worms were present at the fourth larval stage in responders (3.2%) than non-responders (0.8%). The ratio of fifth/adult stage males to females was very similar in both responders and non-responders (1:1.07 and 1:1.04, respectively). Fifth/adult stage female numbers correlated strongly with day 60 specific egg counts in responders and non-responders (r=0.88 and r=0.96, respectively). Female *T. vitrinus* were considerably more fecund in non-responder than responder bucks (0.076 ± 0.004 and 0.025 ± 0.010, respectively).
Figure 4.8. Individual responder and non-responder abomasal worm burdens.

Figure 4.9. Individual responder and non-responder intestinal worm burdens.
Table 4.4. Mean (± SEM) *T. vitrinus* worm burdens.

<table>
<thead>
<tr>
<th>Animal</th>
<th>X L₃ Early L₄</th>
<th>Mid L₄</th>
<th>Late L₄</th>
<th>Stage 5/Adult Male</th>
<th>Stage 5/Adult Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responders</td>
<td>0 0 50 65 1720 1845</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3680* (± 16) (± 22) (± 572) (± 816) (± 1299)</td>
</tr>
<tr>
<td>Non-responders</td>
<td>0 0 75 4312 4488</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8875* (± 43) (± 765) (± 654) (± 1240)</td>
</tr>
</tbody>
</table>

*= significantly different (p<0.05)

Table 4.5. Mean (± SEM) percentage composition of intestinal worm burdens.

<table>
<thead>
<tr>
<th>Animal</th>
<th>X L₃ Early L₄</th>
<th>Mid L₄</th>
<th>Late L₄</th>
<th>Stage 5/Adult Male</th>
<th>Stage 5/Adult Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responders</td>
<td>0.0 0.0 1.4 1.8 46.7 50.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-responders</td>
<td>0.0 0.0 0.0 0.8 48.6 50.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total worm burdens (*T. circumcincta* plus *T. vitrinus*) were not related to mean FEC between days 21-60 in responders, but were negatively associated with mean non-responder counts ($r = -0.75$). Combining the responder and non-responder values into a single group produced a correlation estimate between mean post-challenge egg count and total worm burden of $r = 0.69$. Similarly, combining responder and non-responder data gave an estimated correlation between total fifth/adult stage females and mean FEC of $r = 0.73$. 

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4.3.5 Eosinophil counts

The mean (± SEM) peripheral eosinophil counts for the 5 responder and 4 non-responder bucks following trickle challenge in 1993 are shown in Figure 4.10. Circulating eosinophil numbers increased more rapidly in responders, reaching a significantly elevated peak between days 29-36 after first infection (p<0.05). This peak response was significantly greater than that seen in non-responders (p<0.05). Non-responder peak eosinophil levels occurred between days 36 and 46 but were not significant. There was a strong negative correlation between mean non-responder FEC post-challenge and circulating eosinophil numbers (r = -0.90) though this was not reflected in responders.

The peripheral eosinophil counts of the 3 breeding responders after challenge in 1993 and 1994 are compared in Figure 4.11. It is clear that there was considerable individual variation between years, with a much higher and considerably earlier peak response in the second year, although the differences were not significant.

Though there were obvious differences between the eosinophil responses of the 3 breeding responders in 1993 and 1994 the response was much more similar to that seen in the responder rather than the non-responder bucks. The results of pooling the data collected over the 2 years of the study are shown in Figure 4.12. The numbers of circulating eosinophils increased significantly faster in responders than in non-responders to reach significantly elevated peak levels on day 28 after first infection (p<0.05). Non-responder counts were initially lower and increased only slowly to a non-significant maximum value on day 36. There were no significant differences in total white cell numbers over the period of challenge.
Figure 4.10. Mean (± SEM) responder and non-responder peripheral eosinophil counts following trickle challenge, 1993.

Figure 4.11. Mean (± SEM) peripheral eosinophil counts for the 3 breeding responder bucks following trickle challenge in 1993 and 1994.
Figure 4.12. Mean (± SEM) responder and non-responder peripheral eosinophil counts following trickle challenge in 1993 and 1994.

High average eosinophil counts were associated with high *T. circumcincla* numbers in responders \( (r = 0.69) \), but strongly correlated with low abomasal burdens in non-responders \( (r = -0.99) \). In both groups there was no apparent association between blood eosinophil numbers and intestinal worm burdens. Combining responder and non-responder data showed there to be a negative correlation between mean peripheral eosinophil levels and mean FEC post-challenge \( (r = -0.83) \). There was no apparent association with total worm burden.

Mean (± SEM) abomasal and intestinal tissue eosinophil numbers are shown in Tables 4.6 and 4.7. Abomasal and small intestinal tissue eosinophil numbers were significantly elevated in responders compared with non-responders \( (p<0.05) \), (see Tables 4.6 and 4.7). There was very little apparent correlation between numbers of abomasal or intestinal tissue eosinophils and worm burden.
Table 4.6. Mean (± SEM) abomasal mucosal mast cell (MMC), globule leukocyte (GL), % of MMCs as GLs (% GL) and tissue eosinophil (Eos) numbers.

<table>
<thead>
<tr>
<th>Animal</th>
<th>MMC</th>
<th>GL</th>
<th>% GL</th>
<th>Eos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responders</td>
<td>35</td>
<td>15</td>
<td>30.0</td>
<td>14.5*</td>
</tr>
<tr>
<td></td>
<td>(± 6)</td>
<td>(± 4)</td>
<td>(± 1.2)</td>
<td></td>
</tr>
<tr>
<td>Non-responders</td>
<td>32</td>
<td>8</td>
<td>20.0</td>
<td>5.7*</td>
</tr>
<tr>
<td></td>
<td>(± 5)</td>
<td>(± 4)</td>
<td>(± 1.9)</td>
<td></td>
</tr>
</tbody>
</table>

* = significantly different (p<0.05)

Table 4.7. Mean (± SEM) intestinal mucosal mast cell (MMC), globule leukocyte (GL), % of MMCs as GLs (% GL) and tissue eosinophil (Eos) numbers.

<table>
<thead>
<tr>
<th>Animal</th>
<th>MMC</th>
<th>GL</th>
<th>% GL</th>
<th>Eos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responder</td>
<td>33</td>
<td>20</td>
<td>37.7</td>
<td>29.9*</td>
</tr>
<tr>
<td></td>
<td>(± 5)</td>
<td>(± 6)</td>
<td>(± 4.2)</td>
<td></td>
</tr>
<tr>
<td>Non-responders</td>
<td>42</td>
<td>23</td>
<td>35.4</td>
<td>12.5*</td>
</tr>
<tr>
<td></td>
<td>(± 6)</td>
<td>(± 5)</td>
<td>(± 3.9)</td>
<td></td>
</tr>
</tbody>
</table>

* = significantly different (p<0.05)

4.3.6. Mast cell responses

The mean numbers of mucosal mast cells and globule leucocytes calculated from abomasal and jejunal sections are shown in Table 4.6 and Table 4.7, respectively. No significant differences were apparent in the numbers of MMCs or GLs, or in the proportion of total mast cells (MMC + GL) present as GLs from either...
organ in responders and non-responders. There was little evidence of a consistent relationship between abomasal or small intestinal MMC and GL numbers, or the proportion of GLs present, and *T. circumcincta* or *T. vitrinus* worm burden.

### 4.4 Discussion

These results suggest that it is possible, under the temperate climatic conditions encountered in this study, to select intact male cashmere-producing goats for increased resistance to gastrointestinal nematode infection using simple parasitological criteria, as individual responsiveness appeared to be a relatively consistent and non site-specific characteristic.

Responder and non-responder FECs at pasture were significantly different both from each other and the herd mean, these differences remaining consistent over time in response to both artificial/natural and natural reinfection challenge. The significant FEC repeatability estimates obtained over the course of the field study are encouraging as they suggest that individual resistance to nematode infection is a relatively stable phenomenon.

One of the goals of this study was to ascertain whether responsive individuals could be identified using the simplest parasitological parameter; faecal egg count following artificial and natural challenge infection. Previous ovine selection studies in Australia and New Zealand have used FEC as the simplest parameter with which to measure host responsiveness though the criteria vary. Selection programmes in New Zealand have identified Romney lambs resistant to *Trichostrongylus* spp. and *Teladorsagia* spp. infection following natural pasture infection interrupted by anthelmintic treatment (Baker *et al.*, 1990). Under these conditions post-treatment
egg counts appeared to provide the best indication of host resistance as they reflected the role of acquired immunity (Baker et al., 1990). In contrast, Australian studies have focused upon responsiveness to artificial infection; vaccination and homologous challenge of Merino lambs in the *Trichostrongylus* selection lines (Windon and Dineen, 1984) and single challenge superimposed on grazing lambs in the *Haemonchus* programmes (Albers et al., 1984; Woolaston, 1990).

Though FEC is the most utilised measure of host resistance to infection as counts are relatively cheaply and easily performed, it does possess several drawbacks. Most importantly, although egg count correlates well with current *H. contortus* (Roberts and Swan, 1981) and trichostrongyle burdens (Bisset et al., 1991), it is a much less reliable indicator of worm numbers for species such as *Teladorsagia* where egg output follows a markedly stereotypic pattern (Michel, 1969; Jackson and Christie, 1979). However FEC does provide an important measure of the level of pasture contamination (Albers and Gray, 1986).

Though the bucks were known to be harbouring anthelmintic resistant nematodes (Jackson et al., 1992a) their presence when housed at Moredun caused more problems than anticipated. In all, the bucks were housed for 4½ months, and treated with fenbendazole, ivermectin and a combination of fenbendazole, levamisole and ivermectin before finally being cleared out with 10 consecutive daily treatments of ivermectin. A pilot study conducted at the Moredun Institute using does naturally infected with similar anthelmintic resistant nematodes, found that 10 consecutive daily treatments of ivermectin at 400 μg kg⁻¹ body weight successfully removed all worm stages (Jackson, unpublished data). However the bucks used in this study provided clear evidence of the long term potential that unresponsive
animals have to disseminate drug resistant populations of gastrointestinal nematodes. Although the initial fenbendazole and ivermectin treatments reduced egg counts in both groups, responder counts remained low (mean 30 epg) during the following 15 weeks whereas non-responder counts increased rapidly and remained at higher levels thereafter (mean 420 epg). The results from this study provide an illustration of the effect that non-responsive animals may have in the incrementation of drug resistant populations. During the attempts to remove these resistant populations the non-responders were estimated to have shed more than 10 times the numbers of eggs that the responder animals passed in their faeces. The increased egg counts that were seen post-treatment in the non-responders are probably not simply attributable to the survival of drug resistant adult populations but are also, to some extent, influenced by short term effects upon fecundity and by additional recruitment to the adult population. There can be no doubt that some females survived treatment since the counts remained positive during the immediate post-treatment period. Suppression of egg laying following ivermectin treatment has also been recorded in a multiple resistant strain of *Teladorsagia* isolated from goats at Sourhope (Jackson 1993). The complex population structures that are found in naturally infected animals may also have played some part in the increases in egg count that were seen post-treatment. Previous studies in which naturally infected Scottish cashmere does have been killed at the end of the grazing season at Sourhope have shown that some individuals may harbour very large numbers of inhibited/retarded *Teladorsagia* fourth stage larvae (Jackson unpublished data). It is entirely possible that in this study inhibited and immature stages may have provided a population from which new adult stages were recruited during the post-treatment period. Anthelmintic treatments which remove
the bulk of the adult population may, in the medium term, exacerbate this effect since they may result in a period of reduced antigenic stimulation and hence reduced host immunoresponsiveness. Studies in Australia (Barger 1988a) have shown that lambs given one or more anthelmintic treatments whilst grazing took considerably longer to mount an effective immune response against *H. contortus* compared to untreated individuals. Recent studies in New Zealand have shown direct effects upon the *in vitro* responses of ovine lymphocytes exposed *in vivo* to either levamisole (Cabaj, Stankiewicz, Jonas & Moore 1995) or oxfendazole (Stankiewicz, Cabaj, Jonas, Moore & Chie 1994). Changes in fecundity have been recorded in previous studies at Moredun which have shown a marked increase in the faecal egg counts of naturally infected lambs on housing. This increase has been attributed to changes in immunoresponsiveness that appear to result from the removal of larval antigenic stimulation (R.L. Coop, personal communication). Such changes are almost certainly important in explaining the magnitude and persistence of egg production in the non-responsive goats. The extended survival potential of these drug resistant histotrophic stages in goats poses a considerable threat as far as the dissemination of anthelmintic resistance through animal movement is concerned. This is simply because one cannot assume that animals with negative egg counts are in fact carrying negligible worm populations.

The selective breeding programme required the use of several of the responder bucks over two breeding seasons in order to minimise single sire effects. The egg count and eosinophil data obtained from the same responder males over two years illustrate the importance of seasonal effects upon the magnitude and rate of response. The overall mean faecal egg counts of these animals were significantly
lower during the second season (1994) and there was a more marked eosinophilia. Despite these apparent differences there was, however, no evidence suggesting that responsiveness *per se* was influenced by seasonal differences in parasitology, nutrition, and/or climate.

A potential shortfall of FEC as an indicator of nematode burden is that the numbers of eggs shed per gramme of faeces is dependent on the diluting effect of total faecal output which is in turn influenced by faecal consistency and body size. Studies in New Zealand have suggested that one of the unwelcome side-effects of selecting for responsiveness in sheep may be an increased incidence of fleece dags (Watson *et al.*, 1986). The association between increasing host-resistance and an increasing dag score may be less important in goats reared in temperate climates since fibre losses through dagging are generally lower in goats than sheep and the risks of myiasis are generally much lower in temperate zones. In the current study softening of the faeces was most apparent in the bucks in the post-turnout period and again at housing. However these differences were relatively minor, in most cases faeces were passed as an unformed mass rather than as pellets. Recent ovine studies have suggested that diarrhoea and dag score may be indicative of the level of larval challenge and host inflammatory responses rather than current worm burden (Larsen *et al.*, 1994; Douch *et al.*, 1995a; Suttle and Brebner, 1995).

The expression of host immunity to parasitic challenge is highly dependent on various external factors, notably plane of nutrition (Wagland *et al.*, 1984) and previous exposure to infection (Gill, 1991). Previous studies on the development of immunity in young lambs to *T. colubriformis* infection have shown that though levels of protein intake had little effect on worm establishment and initial fecundity,
supplemented animals were better able to regulate their adult worm burdens (van Houtert et al., 1995). It is thus encouraging that individual bucks were responsive whilst on pasture and when housed and maintained on a good plane of nutrition. Previous exposure to gastrointestinal nematode infection may be the most important factor in determining host responsiveness. Studies conducted by Gray et al. (1990) have suggested that the expression of immunity in young lambs may be heavily influenced by environmental factors acting several months earlier. However, studies in New Zealand have found that, unlike young lambs, young goat kids appear unable to develop any observable resistance to infection in their first year (Brunsdon, 1986). Since the bucks used in this study were drawn from 12 different sources it is impossible to say with any certainty what effect, if any, such influences may have had on the findings of this study. The situation is further complicated by the bucks all being at least 2 years old at the start of the study, and the study itself being conducted over a 2 year period.

Post-challenge worm burdens confirmed the segregation of responder and non-responder bucks. Responder and non-responder abomasal and intestinal worm burdens showed considerable differences, with responders harbouring fewer adults and a much higher proportion of larvae exhibiting some retardation of development. Acquired immunity is often expressed through reductions in larval establishment and development, with host induced effects on the sex ratio and fecundity of established worms (Dineen and Windon, 1980). Previous studies investigating the development of resistance in lambs to *T. vitrinus* and *T. circumcincta* infection have suggested that one of the first manifestations of acquired immunity is a reduction in the rate of larval development (Seaton et al., 1989 a, b). Effects on worm fecundity have been
reported from studies on the acquisition of immunity to *T. colubriformis* and *H. contortus* infection in lambs (Dineen and Windon, 1980; Gill *et al.*, 1991). In the current study there were no differences in sex ratios between responders and non-responders but there did appear to be an effect on fecundity for *T. vitrinus*. Although individual female *T. circumcincta* shed similar numbers of eggs in responders and non-responders, female *T. vitrinus* were three times more fecund in non-responders. Specific egg counts conducted at time of slaughter also correlated with adult female numbers in both responder and non-responder bucks. There was no correlation between mean responder egg count post-challenge and worm burden on day 60, though an inverse relationship was recorded for non-responders. However combining the responder and non-responder counts into a single group showed mean post-challenge FEC to be positively associated with worm burden.

Since grazing animals rarely, if ever, encounter infection with a single gastrointestinal nematode species, to be viable a selective breeding programme requires host resistance to be expressed against a number of unrelated species. The results of the present study suggest that increased responsiveness was not restricted to a single species or site of infection. This is in agreement with both ovine (Windon, 1990) and small animal studies (Biozzi, 1982) which have suggested that resistance to infection may be polyspecific. Previous studies have shown that selection for increased resistance to *T. colubriformis* infection confers a moderate degree of cross-protection following vaccination and artificial challenge with *H. contortus* (Windon *et al.*, 1987). In addition high responder lambs from the *Trichostrongylus* selection line had significantly reduced egg counts following artificial challenge with *T. axei*, *T. rugatus* and *T. colubriformis* and natural pasture infection with *Trichostrongylus*.
spp. and *Ostertagia* spp. (Windon and Dineen, 1984; Windon *et al.*., 1987). Similarly, high responder lambs from the Australian *Haemonchus* lines displayed increased responsiveness towards heterologous *T. colubriformis* infection (Woolastion, 1990). However, studies have shown that the differences between resistant and susceptible individuals from the *Trichostrongylus* and *Haemonchus* lines following heterologous *H. contortus* or *T. colubriformis* infections, respectively, are not as great as after homologous challenge with which the lines had been selected (Windon, 1990; Woolastion, 1990). New Zealand studies demonstrated that Romney lambs selected for high or low responsiveness to naturally acquired *Trichostrongylus* spp. and *Teladorsagia* spp. infection were equally susceptible to natural *H. contortus* infection (Baker *et al.*, 1990).

The identification of animals at either end of the extremes of responsiveness should, in theory, provide groups containing relatively homogenous animals ideally suited for studies on parasite population regulation and the immunological responses associated with infection. Immunoregulatory mechanisms are notoriously complex involving a number of specific key components, and moreover responses inevitably encompass both quantitative and qualitative elements (Miller, 1984; Rothwell, 1989).

The association between gastrointestinal helminthiasis and peripheral and tissue eosinophilia is well documented and has recently been reviewed (Jones, 1993). In the current study, peripheral eosinophil levels displayed marked, though non-significant, differences within the three breeding responders between challenge in 1993 and 1994. When the results were combined, responder blood eosinophil levels post-challenge were seen to increase significantly faster and reach a significantly
higher peak than recorded from non-responders. The high peripheral eosinophil numbers detected in responder bucks is in agreement with previous ovine studies which have suggested that increased eosinophilia may reflect the expression of host responsiveness rather than levels of helminth infection (Dawkins et al., 1989). Laboratory studies have suggested that the speed of eosinophil response may be an important factor in determining the resistance status of the host (Handlinger and Rothwell, 1981; Wakelin and Donachie, 1983; Lawrence and Pritchard, 1994). Responder and non-responder tissue eosinophil numbers were inversely related to worm numbers suggesting a potential role for eosinophils in the immunoregulatory processes if not a direct role in the effector mechanisms. The relationship between tissue eosinophil levels and resistance is unclear (Rothwell, 1989). An inverse relationship between tissue eosinophil numbers and the resistance status of sheep infected with *T. colubriformis* was recorded by Dineen and Windon (1980), but this conflicts with the positive relationship reported by Dineen et al. (1978), and the lack of an apparent relationship described by Gregg et al. (1978).

Mucosal mastocytosis and globule leukocyte and goblet cell hyperplasia are characteristic of gastrointestinal nematode infection in ruminants (Miller, 1984). Globule leukocytes are degranulated mast cells (Huntley et al., 1984), whose presence has been closely associated with host responsiveness (O’Sullivan and Donald, 1973; Stankiewicz et al., 1993). In the current study there were no significant differences in abomasal or intestinal MMC or GL numbers between responders and non-responders, although non-responders had one-third more intestinal MMCs than responders. There was very little correlation between MMC levels and *T. vitrinus* burdens within either group, though they were weakly
positively and negatively related to responder and non-responder abomasal burdens, respectively. Globule leukocytes were twice as numerous in responder abomasal tissues as those of non-responders, though they were slightly more common in the intestinal tissues of non-responders. The tendency among both responders and non-responders was for GL numbers to be negatively associated with worm burden. It is possible that small intestinal mast cell responses in this study may be confounded by the effects of exposure to other intestinal pathogens (Huntley *et al.*, 1985). Although direct comparison with the does in Chapter 3 is not possible due to differences in previous exposure, age and experimental design, it is notable that MMCs were considerably more numerous in the bucks in this study. As the does and bucks had very similar GL numbers, the proportion of mast cells present as GLs was very much lower in the latter. It is unclear whether this is attributable to sex-related differences in response or between-experiment differences in infection. Since there was little evidence of any correlation between MMC levels and host resistance in the current study, any role for MMCs in responsiveness in male goats is more likely to be qualitative than quantitative.

These results clearly demonstrate the potential for using simple parasitological criteria to identify intact male goats of high and low responsiveness to gastrointestinal nematode infection. Faecal egg counts following artificial and natural infection proved to be a reliable indicator of host resistance status and were supported by egg counts and worm burdens resulting from trickle challenge. Individual responsiveness was found to be a stable characteristic over time with high repeatability estimates for combined responder and non-responder FECs following artificial/natural infection, housing and artificial trickle challenge. The fact that the
responsiveness of Scottish cashmere bucks was not species or site limited is an important finding and lends support for the use of selective breeding as a means of reducing herd susceptibility and hence the reliance upon routine chemotherapy.
Chapter 5 - Responses of female Scottish cashmere goats to gastrointestinal nematode infection
5.1 Introduction

The prevalence of anthelmintic resistance among the gastrointestinal nematodes of small ruminants (Jackson, 1993) and its effects upon production (Holmes, 1985) are of major concern to small-ruminant producers. A combination of differences in drug pharmacokinetics (Sangster et al., 1991) and an increased susceptibility to nematode infection (McKenna, 1984; Watson and Hosking, 1989) may lead to grazing goats being an important source of drench resistant parasites.

A number of ovine studies have investigated the potential for exploitation of the high degree of between- (Stewart et al., 1937), and within-breed (Albers et al., 1984) variation in host responsiveness to nematode infection. Selective breeding programmes have been implemented successfully in Australia and New Zealand to produce lines of Merino and Romney sheep with increased resistance to nematode infection (Windon, 1990). Although few studies have examined the genetic resistance of goats to nematode infection previous studies have suggested that genetic variation in resistance does exist (Preston and Allonby, 1978; Richard et al., 1990). However, a recent tropical study reported an absence of genetic variation or repeatability in the FECs of goats following nematode infection (Woolaston et al., 1992b).

Eosinophilia, mucosal mastocytosis and globule leukocyte hyperplasia have been closely associated with ovine responsiveness (Rothwell, 1989). It has also been demonstrated that local mucosal responses involving the increased production of lymphocytes, lymphoblasts and IgA-containing cells detectable in efferent lymph may be an important constituent of the immunological responses of sheep exposed to infection with *T. colubriformis* (Adams and Cripps, 1977; Adams et al., 1980) and *T.*
*circuncincta* (Smith *et al*., 1983b; Smith *et al*., 1984a). A number of studies have investigated the possibility of using peripheral responses as predictive markers which may be associated with an increased resistance to gastrointestinal nematode infection (see Chapter 1). As part of the current studies the possibility of using a novel antigen (in this case chicken albumin) as an indicator of host resistance was assessed.

These studies investigate the responsiveness of a breeding group of Scottish cashmere does over a 2 year period. The results enable a comparison of the responses of breeding female and male goats (see Chapter 4). In addition they provide original data concerning the local immune responses as measured at the site of infection and also evidence as to the specificity of increased immunological responsiveness.

### 5.2 Materials and Methods

#### 5.2.1 Animals

The initial study herd, comprising 95 2-6 years-old Scottish cashmere does was drawn randomly from does grazed at the Sourhope farm in October 1992. In October of each subsequent year approximately one-third of the breeding herd were replaced with an equal number of younger does. In the second season these replacements were drawn from fibre-line does on the property, but in the third and fourth seasons females born into the helminth-line in 1993 and 1994, respectively, were used. All animals had been exposed to natural infection with the nematode species used in these studies.
5.2.2 Breeding programme

In late autumn the does were mated with the selected top responder bucks. In the first two years of the breeding programme (1992-93 and 1993-94) these bucks came from those animals described in the previous chapter (Chapter 4). However in subsequent years bucks were selected from the yearling males of the helminth-line.

5.2.3 Identification of responder and non-responder does

An outline of the timetable used for the pasture grazing studies conducted between 1992 and 1994 are shown in Table 5.1.

**Table 5.1. Outline of experimental timetable used during the pasture grazing studies with the does 1992-1994.**

<table>
<thead>
<tr>
<th>Month</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>October 1992</td>
<td>95 2-4 years old does gathered into helminth-line herd.</td>
</tr>
<tr>
<td></td>
<td>Does bred to F8002 and C9053</td>
</tr>
<tr>
<td>April-June 1993</td>
<td>1993 first-generation kids born</td>
</tr>
<tr>
<td>November 1993</td>
<td>Selected responders and non-responders housed at Moredun for</td>
</tr>
<tr>
<td></td>
<td>25%-30% of helminth-line does culled and replaced with an equal</td>
</tr>
<tr>
<td></td>
<td>number of younger animals. Helminth-line does bred with F8002, D1195 and</td>
</tr>
<tr>
<td></td>
<td>H8088.</td>
</tr>
<tr>
<td>November 1994</td>
<td>Selected responders and non-responders housed at Moredun for</td>
</tr>
<tr>
<td></td>
<td>artificial challenge in 1995.</td>
</tr>
</tbody>
</table>

Does in both seasons were segregated into responders and non-responders on the basis of their FECs following exposure to natural infection at grazing. Field studies
were conducted over a 12 month period, commencing in November, on pasture contaminated predominately with *T. circumcincta* and *T. vitrinus* infective larvae (Jackson *et al.*, 1992b). Individual rectal faecal samples were obtained every 5 weeks prior to anthelmintic treatment. Samples were not collected around the time of kidding to avoid stressing the does unduly. The does were treated with levamisole (12 mg kg⁻¹) in 1992-93 and ivermectin (200 µg kg⁻¹) in 1993-94.

Individual FECs were ranked throughout the season, the lowest count being assigned a ranking of 1 and the highest a value of 95. At the end of the study period a cumulative ranking was obtained. Responders were deemed to be those with the lowest cumulative rankings, while those with the highest rankings were classified as non-responders. In this way the 8 most and 8 least responsive does, responders and non-responders respectively, were selected for further study. All selected animals had single live offspring at grazing.

5.2.4 Faecal egg counts

Faecal samples were scored and egg counts performed using a modified flotation technique as previously described (Chapter 2). Specific egg counts were conducted on samples collected from does on pasture as described in Chapter 2.

5.2.5 Artificial trickle challenge infection

At the end of each of the two grazing seasons the selected responder and non-responder does were housed at Moredun to undergo an artificial challenge infection. The does were housed in a single group on straw covered concrete floors under conditions designed to minimise the risk of accidental infection and given 10 consecutive daily treatments of ivermectin (400 µg kg⁻¹). While housed the does were maintained on a diet of ESCA nuts (East of Scotland College of Agriculture,
14% crude protein) and hay and water *ad libitum*. An outline of the experimental protocol used with the does following housing in both 1993 and 1994 is shown in Table 5.2.

**Table 5.2. Outline of experimental timetable used in the artificial challenge of housed does in both 1993 and 1994.**

<table>
<thead>
<tr>
<th>Day After First Infection</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>-31</td>
<td>Selected does housed at Moredun.</td>
</tr>
<tr>
<td>-30 to -21</td>
<td>Given 10 consecutive treatments with ivermectin.</td>
</tr>
<tr>
<td>0 to 24</td>
<td>Trickle challenge infection.</td>
</tr>
<tr>
<td>60</td>
<td>Treated with fenbendazole.</td>
</tr>
<tr>
<td>64</td>
<td>Cannulation of gastric lymphatic vessels.</td>
</tr>
<tr>
<td>67</td>
<td>Given single challenge of 50,000 <em>T. circumcincta</em>.</td>
</tr>
<tr>
<td>77</td>
<td>Killed for post-mortem analysis.</td>
</tr>
</tbody>
</table>

A mixed oral challenge of 2000 *T. circumcincta* and 1000 *T. vitrinus* L₃ per day, 5 days per week for 4 weeks commenced 21 days after the last anthelmintic treatment. Both laboratory strains were known to be susceptible to drugs in all three broad spectrum anthelmintic families. Faecal egg counts were conducted twice weekly up to day 60 after first infection.

In November 1993, at the end of the first grazing season, the selected responder and non-responder does were housed together with 10 does randomly selected from those grazed at Sourhope. These unselected animals were of an equivalent age and had had similar exposure to gastrointestinal nematode infection on pasture at Sourhope.
5.2.6 *Anthelmintic control study*

Two of the unselected does housed and treated in November 1993 were killed prior to commencement of the trickle challenge to determine the efficacy of the course of anthelmintic treatment. These animals had been exposed to natural pasture infection at Sourhope and harboured patent nematode burdens as established by FEC.

5.2.7 *Single challenge infection*

On day 60 after commencement of the trickle challenge the does were treated with anthelmintic (fenbendazole, 5 mg kg\(^{-1}\)) and 7 days later (day 67) given a single oral challenge with 50,000 *T. circumcincta* L\(_3\). Ten days post-infection (day 77) the does were killed for post-mortem analysis as described earlier (Chapter 2). One of the selected responders in the first year's study died from a cause other than parasitism prior to single challenge leaving a study group of 7 animals.

5.2.8 *Eosinophil counts*

Venous blood samples were taken into EDTA-containing vacutainers weekly up to day 60 following commencement of trickle infection and also on day 66 prior to single challenge. Following challenge, venous blood was collected at 2 day intervals up to the time of slaughter. Peripheral eosinophil levels were determined after fixing in Carpentier's stain as described (Chapter 2). Total white cell counts were conducted using a model ZM Coulter Counter (Coulter Electronics Ltd., Luton, UK).

5.2.9 *Post-mortem analysis*

An abomasal saline wash and digest were performed for each individual and the numbers and stage of larval development of the worms recovered from 2%
aliquots determined as described previously (Chapter 2). Immediately after slaughter excised sections of abomasal folds were processed for enumeration of mucosal mast cells, globule leukocytes, tissue eosinophils and SMCP concentrations.

5.2.10 Antigenic stimulation

On day 0 of the trickle infection in 1995 the responder and non-responder does were given a subcutaneous injection with chicken albumin (Sigma) dissolved 2 mg per ml in 10% alhydrogel (3% Al(OH)₃, Superfoss). Animals were injected at 1ml per 100 kg bodyweight. An identical secondary challenge was administered 3 weeks later.

5.2.11 Lymphatic cannulation

On day 64, 3 days prior to single _T. circumcincta_ challenge, the does underwent surgery for cannulation of the common gastric lymph duct as described previously (Chapter 2). Where available efferent lymph was collected daily and analysed as described in Chapter 2.

5.2.12 Statistical analysis

The arithmetic mean (± SEM) is quoted in all instances. Statistical analyses were performed using the untransformed mean data for each individual and the effect of group tested with the Mann-Whitney test. Faecal egg count repeatability estimates were obtained by calculating the correlation between adjacent log₁₀(x + 1) transformed samples within each group. Analyses of estimates of FEC repeatability were conducted using the 1-sample Wilcoxon test.
5.3 Results

5.3.1 Pasture faecal egg counts

Specific faecal egg counts

The numbers of *T. circumcincta* and *T. vitrinus* eggs in faecal samples taken from does on pasture between September 1993 and December 1995 are shown in Figure 5.1. There was evidence of two waves of *T. circumcincta* infection, firstly in late spring/early summer and again in late summer/autumn. *T. vitrinus* numbers increased in spring with evidence of a slight rise in summer. *T. vitrinus* eggs predominated over winter.

![Figure 5.1 T. circumcincta and T. vitrinus egg counts of does on pasture.](image)

Identification of responder and non-responder does in 1992-93

The mean (± SEM) FECs of the selected 8 responder and 8 non-responder does together with the herd mean are shown in Figure 5.2. Retrospective analysis showed that responders had shed significantly fewer eggs on pasture than had non-responders (52 ± 5 epg compared to 234 ± 18 epg, respectively, p<0.001). Mean
Figure 5.2. Mean (± SEM) FECs of selected 8 responder and 8 non-responder does together with the herd mean following natural challenge, 1992-93.

Figure 5.3. Mean (± SEM) FECs of selected 8 responder and 8 non-responder does together with the herd mean following natural challenge, 1993-94.
responder and non-responder counts were significantly different than the herd mean (124 ± 7 epg, \( p<0.001 \)). Faecal egg counts in all 3 groups increased around parturition though this was most marked in non-responders. Post-kidding egg counts were significantly higher than those obtained in the later stages of pregnancy for both the herd mean (\( p<0.001 \)) and selected non-responders (\( p<0.05 \)). There was no equivalent significant increase in responder egg count. Though there was a certain degree of variability in pasture rankings, selected responders and non-responders were consistently ranked within the 15 most or 15 least responsive individuals, respectively. FEC repeatability estimates were significantly greater than zero for the entire herd (0.19 ± 0.05, \( p<0.01 \)), but not for the selected responder or non-responder does.

*Identification of responder and non-responder does in 1993-94*

The mean (± SEM) egg counts of the selected 8 responder and 8 non-responder does from the second year’s grazing study are shown together with the mean herd counts in Figure 5.3. Mean responder FEC (69 ± 8 epg) was significantly lower than that of non-responders (292 ± 25 epg, \( p<0.001 \)) and both were significantly different from the herd mean of 156 (± 7) epg (\( p<0.001 \)). The postparturient rise in FEC was most pronounced in selected non-responders with no evidence of any similar increase in responder egg count. Selected responder and non-responder does were respectively ranked among the 15 most or least responsive individuals throughout the grazing study. Estimates of repeatability of faecal egg count were significantly greater than zero for the herd mean (0.26 ± 0.05, \( p<0.01 \)) but not for either the responder or non-responder does.
**Faecal consistency scores**

Non-significant differences were apparent in the faecal consistency scores of the does on pasture in both 1992-93 and 1993-94 (Figures 5.4 and 5.5, respectively). Faecal consistency score fell considerably in all groups around the time of the PPR before returning to pre-kidding levels in late autumn.

### 5.3.2 Housed faecal egg counts

**Artificial trickle challenge 1994**

The mean (± SEM) FECs of the 8 responder, 8 non-responder and 8 unselected does following trickle infection are shown in Figure 5.6. Over the period of artificial challenge responders had a mean egg count of 24 (± 9) epg, significantly lower than that of non-responder (252 ± 70 epg, p<0.001) and unselected does (128 ± 25 epg, p<0.001). Mean FEC repeatability estimates were 0.70 (± 0.05), 0.70 (± 0.06) and 0.74 (± 0.07) for responder, non-responder and unselected does, respectively; all significantly better than zero (p<0.001). Combining responder and non-responder counts into one group gave a FEC repeatability estimate between mean natural and artificial infection of 0.78. There were no differences in faecal consistency between or within groups following infection.

**Artificial trickle challenge 1995**

The mean (± SEM) egg counts of the selected 8 responder and 8 non-responder does following trickle infection in 1995 are shown in Figure 5.7. Between days 21 and 60 after first infection mean responder FEC (165 ± 32 epg) was significantly lower than that of non-responders (290 ± 29 epg, p<0.01). Responder egg counts were significantly lower than those of non-responders on days 21, 24, 31, 36 and 59 after first infection (p<0.05). Faecal egg count repeatability estimates were
Figure 5.4. Mean (± SEM) faecal scores of samples collected from the does on pasture, 1992-93.

Figure 5.5. Mean (± SEM) faecal scores of samples collected from the does on pasture, 1993-94.
Figure 5.6. Mean (± SEM) FECs of responder, non-responder and unselected does following trickle challenge, 1994.

Figure 5.7. Mean (± SEM) FECs of selected responder and non-responder does following trickle challenge, 1995.
significant for both responders and non-responders (0.83 ± 0.03 and 0.45 ± 0.09, respectively, \( p<0.001 \)). Combining the counts into a single group gave a repeatability estimate for mean FEC on pasture and after artificial trickle infection of 0.73. There were no differences in faecal consistency between or within groups after infection.

5.3.3 Post-challenge worm burdens

*T. circumcincta burdens in 1994*

The population structure of individual *T. circumcincta* burdens recovered on day 10 post-challenge are illustrated in Figure 5.8. The mean numbers and proportions of established worms recovered at each stage of larval development are shown in Tables 5.3 and 5.4 respectively.

Mean responder burden (12,686 ± 3946) was approximately three-quarters that of non-responder and unselected does (17,744 ± 1556 and 17,613 ± 2473, respectively) though these differences were not statistically significant. There were considerable, though non-significant, differences between the 3 groups in larval development, responders having a much higher proportion of established larvae present as early and mid L4, (60%), compared to non-responder and unselected does (both approximately 40%). As only one animal harboured any fifth-stage larvae the proportion of established worms present at this stage in responders was significantly lower than in non-responders (\( p<0.05 \)). This individual also harboured a large number of late fourth-stage larvae and had the largest burden of any animal in this study (data not shown). In contrast, the majority of established larvae in non-responders and unselected does were present at the late L4 or fifth-stage.

Total abomasal worm burden 10 days after single challenge was positively associated with mean FEC following artificial trickle challenge in both responder
and unselected does ($r=0.82$ and $r=0.64$, respectively) though this was not apparent in non-responders. There was no association between combined responder, non-responder and unselected doe post-challenge FECs and day 10 worm burdens.

*T. circumcincta burdens in 1995*

Individual *T. circumcincta* burdens recovered from the does in 1995 are shown in Figure 5.9. Mean responder and non-responder worm burdens together with the stages of larval development are shown in Table 5.3.

The mean responder ($5838 \pm 2218$) and non-responder ($6344 \pm 2660$) day 10 worm burdens were not significantly different, however responders had a greater proportion of early and mid L4 than did non-responders ($55\%$ and $35\%$, respectively, Table 5.4). There was little evidence of any association between worm burden on day 10 post-challenge and mean egg counts following trickle infection.
**Figure 5.8.** Individual abomasal worm burdens 10 days post-challenge, 1994.

**Figure 5.9.** Individual abomasal worm burdens 10 days post-challenge, 1995.
Table 5.3. *Mean (± SEM) T. circumcincta burdens recovered from does challenged in 1994 and 1995.*

<table>
<thead>
<tr>
<th>Group</th>
<th>Early L₄</th>
<th>Mid L₄</th>
<th>Late L₄</th>
<th>Stage 5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responders</td>
<td>5340</td>
<td>2296</td>
<td>4956</td>
<td>94</td>
<td>12,686</td>
</tr>
<tr>
<td></td>
<td>(± 1709)</td>
<td>(± 960)</td>
<td>(± 3989)</td>
<td>(± 94)</td>
<td>(± 3946)</td>
</tr>
<tr>
<td>Non-responders</td>
<td>5850</td>
<td>1450</td>
<td>8300</td>
<td>2144</td>
<td>17,744</td>
</tr>
<tr>
<td></td>
<td>(± 1777)</td>
<td>(± 617)</td>
<td>(± 2555)</td>
<td>(± 966)</td>
<td>(± 1556)</td>
</tr>
<tr>
<td>Unselected</td>
<td>4892</td>
<td>1069</td>
<td>10,383</td>
<td>1269</td>
<td>17,613</td>
</tr>
<tr>
<td></td>
<td>(± 830)</td>
<td>(± 256)</td>
<td>(± 244)</td>
<td>(± 683)</td>
<td>(± 2473)</td>
</tr>
<tr>
<td>1995</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responders</td>
<td>2067</td>
<td>1146</td>
<td>1712</td>
<td>913</td>
<td>5838</td>
</tr>
<tr>
<td></td>
<td>(± 914)</td>
<td>(± 409)</td>
<td>(± 890)</td>
<td>(± 490)</td>
<td>(± 2218)</td>
</tr>
<tr>
<td>Non-responders</td>
<td>1702</td>
<td>546</td>
<td>2226</td>
<td>1870</td>
<td>6344</td>
</tr>
<tr>
<td></td>
<td>(± 792)</td>
<td>(± 182)</td>
<td>(± 1378)</td>
<td>(± 898)</td>
<td>(± 2660)</td>
</tr>
</tbody>
</table>
Table 5.4. Mean (± SEM) percentage composition of *T. circumcincta* burdens recovered from does challenged in 1994 and 1995.

<table>
<thead>
<tr>
<th>Animal</th>
<th>% of established worms recovered as...</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early L₄</td>
<td>Mid L₄</td>
<td>Late L₄</td>
<td>Stage 5</td>
</tr>
<tr>
<td></td>
<td>(± 9.9)</td>
<td>(± 8.2)</td>
<td>(± 12.2)</td>
<td>(± 0.3)</td>
</tr>
<tr>
<td>Responders</td>
<td>42.1</td>
<td>18.1</td>
<td>39.1</td>
<td>0.7ᵃ</td>
</tr>
<tr>
<td>Non-responders</td>
<td>33.0</td>
<td>8.2</td>
<td>46.7</td>
<td>12.1ᵇ</td>
</tr>
<tr>
<td>Unselected</td>
<td>27.8</td>
<td>6.0</td>
<td>59.0</td>
<td>7.2</td>
</tr>
<tr>
<td>(± 11.4)</td>
<td>(± 1.7)</td>
<td>(± 11.5)</td>
<td>(± 2.7)</td>
<td></td>
</tr>
<tr>
<td>Responders</td>
<td>35.5</td>
<td>19.6</td>
<td>29.3</td>
<td>15.6</td>
</tr>
<tr>
<td>(± 10.3)</td>
<td>(± 5.5)</td>
<td>(± 6.7)</td>
<td>(± 14.6)</td>
<td></td>
</tr>
<tr>
<td>Non-responders</td>
<td>26.8</td>
<td>8.6</td>
<td>35.1</td>
<td>29.5</td>
</tr>
<tr>
<td>(± 9.2)</td>
<td>(± 4.6)</td>
<td>(± 7.6)</td>
<td>(± 10.2)</td>
<td></td>
</tr>
</tbody>
</table>

Figures with different superscripts are significantly different at p<0.05.

5.3.4 Eosinophil counts

*Peripheral eosinophil counts in 1994*

Mean (± SEM) peripheral eosinophil counts for responder, non-responder and unselected does following trickle challenge in 1994 are shown in Figure 5.10. Circulating eosinophil levels increased significantly faster after first infection in responders than either other group (p<0.01). Responders exhibited an 8-fold increase in eosinophil levels to reach a significantly elevated peak on day 21 (p<0.001). Non-responder peripheral eosinophil numbers increased three-fold to a non-significant
peak on day 14 PI. Eosinophil levels in unselected does also increased three-fold by day 42 but this was not significant. Though pre-infection levels were similar, responders had significantly more eosinophils than non-responder and unselected does on days 7 (p<0.01) and 21 (p<0.05), and unselected does on day 14 (p<0.05). Eosinophil levels were similar for all groups by day 35. The trend was for mean combined peripheral eosinophil levels to be negatively associated with mean post-challenge FEC (r=-0.62).

There were no differences in circulating eosinophil numbers in any group over the 10 day period following single artificial challenge (Figure 5.12.)

*Tissue eosinophil counts in 1994*

Although twice as many abomasal tissue eosinophils were recovered from responders and unselected does than non-responders, these differences were not statistically significant (Table 5.5). There was no evidence of an association between abomasal tissue eosinophil numbers and worm burden or composition.

*Peripheral eosinophil counts in 1995*

Mean (± SEM) responder and non-responder peripheral eosinophil counts in 1995 are displayed in Figure 5.11. Circulating eosinophil levels pre-infection were similar in both groups but increased significantly faster in responders (p<0.05). Responders showed a two-fold rise in blood eosinophil numbers to reach a significantly elevated peak on day 21 (p<0.05). Eosinophil levels on day 21 were significantly higher in responder than in non-responder does (p<0.05). There was little change in non-responder eosinophil numbers over the course of infection. From day 36 onwards eosinophil levels were very similar for both groups. There was no
correlation between mean peripheral eosinophil numbers and mean post-challenge FEC.

There were no differences in peripheral eosinophil levels between either of the groups in the 10 days following single *T. circumcincta* infection (Figure 5.13).

*Tissue eosinophil counts in 1995*

Mean (± SEM) responder and non-responder abomasal tissue eosinophil counts in 1995 are shown in Table 5.5. There was no evident relationship between abomasal tissue eosinophil counts and larval numbers or stages of development.

**Table 5.5.** Mean (± SEM) mucosal mast cell (MMC), globule leukocyte (GL), % of MMCs as GLs (%GL), tissue eosinophil (Eos) and SMCP values in 1994 and 1995.

<table>
<thead>
<tr>
<th>Animal</th>
<th>MMC (± SEM)</th>
<th>GL (± SEM)</th>
<th>%GL (± SEM)</th>
<th>Eos (± SEM)</th>
<th>SMCP (μg g⁻¹) (± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responders</td>
<td>75a (±11)</td>
<td>30 (±8)</td>
<td>28.6 (±3.2)</td>
<td>13.8 (±3.4)</td>
<td>1.60 (±0.34)</td>
</tr>
<tr>
<td>Non-responders</td>
<td>43b (±8)</td>
<td>15 (±5)</td>
<td>25.9 (±2.4)</td>
<td>6.4 (±0.6)</td>
<td>1.16 (±0.06)</td>
</tr>
<tr>
<td>Unselected</td>
<td>38b (±7)</td>
<td>8 (±3)</td>
<td>17.4 (±3.5)</td>
<td>15.8 (±0.39)</td>
<td>1.76 (±0.39)</td>
</tr>
<tr>
<td>1995</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responders</td>
<td>33 (±4)</td>
<td>10 (±2)</td>
<td>23.3 (±1.5)</td>
<td>2.5 (±0.17)</td>
<td>0.75 (±0.17)</td>
</tr>
<tr>
<td>Non-responders</td>
<td>33 (±8)</td>
<td>11 (±5)</td>
<td>25.0 (±0.4)</td>
<td>0.5 (±0.17)</td>
<td>0.4 (±0.17)</td>
</tr>
</tbody>
</table>

Figures with different superscripts are significantly different (p<0.05).
Figure 5.10. Mean (± SEM) peripheral eosinophil counts of responder, non-responder and unselected does following trickle challenge, 1994.

Figure 5.11. Mean (± SEM) peripheral eosinophil counts for responder and non-responder does following trickle challenge, 1995.
Figure 5.12. Mean (± SEM) peripheral eosinophil counts of responder, non-responder and unselected does following single challenge, 1994.

Figure 5.13. Mean (± SEM) peripheral eosinophil counts of responder and non-responder does following single challenge, 1995.
5.3.5 Mast cell responses

Mast cell responses in 1994

Mean (± SEM) abomasal mucosal mast cell and globule leukocyte counts obtained for responder, non-responder and unselected does following single challenge in 1994 are shown in Table 5.3. Responders had significantly higher numbers of MMCs than non-responder and unselected does (p<0.05). Although responders had twice as many GLs as did non-responders and almost four-times as many as the unselected does these differences were not significant. Though there were large differences in the numbers of MMCs and GLs, there were no significant differences in the percentage of total mast cells present as GLs between the three groups. There were no associations between MMC or GL numbers and worm burden or composition within each of the groups. However, calculations using the combined data from the three groups showed a tendency for increased MMC and GL numbers to be associated with lower day 10 worm burdens, a greater proportion of retarded larvae and fewer fifth-stage worms.

There were no significant differences in the observed concentrations of SMCP (Table 5.3) between groups. Although the relationship was weak, the tendency was for SMCP concentration to be inversely associated with worm burden and the percentage of stage 5 worms and positively associated with the proportion of early L4.

Mast cell responses in 1995

Group mean (± SEM) abomasal mast cell and globule leukocyte counts from does challenged in 1995 are shown in Table 5.3. The numbers of MMCs, GLs and the percentage of mast cells present as GLs were almost identical between groups.
When the data from the responder and non-responder does were combined into a single group MMC and GL levels tended to be inversely associated with worm burden and the proportion of established larvae present at the fifth-stage.

There were no significant differences in SMCP concentrations between the groups (Table 5.3). There was a weak negative relationship between SMCP concentration and worm numbers and the proportion of fifth-stage worms.

5.3.6 *Chicken albumin stimulation*

The mean (± SEM) antibody responses of responder and non-responder does to primary and secondary challenge with chicken albumin are shown in Figure 5.14. Peak antibody responses were detected one week after secondary challenge and were similar in both groups.

![Graph showing antibody responses](image)

**Figure 5.14** Mean (± SEM) circulating antibody responses of responder and non-responder does following primary and secondary challenge with chicken albumin.
5.3.7 Lymphatic cannulation

Out of the 40 does which underwent surgery in 1994 and 1995, 12 could not be cannulated successfully due to the small size of their lymph vessels. Of the 28 that were successfully cannulated, 19 stopped flowing within 24 hours of surgery and only 4 flowed for more than 48 hours. These 4 does were all challenged in 1994. Due to the small number of animals, individual results are presented. Of the 4 animals to provide lymph data, one was a selected responder (84), one was from the unselected group (A 1149) and two were selected non-responders, (20 and 27). Although there was considerable variation in lymph flow rate throughout the period of study (from a mean of 2.4 ml h⁻¹ for A 1149 to 23.8 ml h⁻¹ in doe 84) there was evidence of a slight increase in lymph flow by day 2 PI. This was most apparent in the selected responder and least in the non-responders.

Mean hourly lymphocyte outputs for each individual are shown in Figure 5.15. There was considerable individual variation in the rate of white cell output, but for each animal there was evidence of increased output post-challenge by days 4 to 5.

A very pronounced lymphoblast response was evident in doe 84 on day 4 (Figure 5.16). Does A1149 and 27 exhibited a very much lower peak lymphoblast output between days 4 and 5 whereas peak lymphoblast output occurred between days 6 and 9 for doe 20. Between the day before and the day after challenge, lymphoblasts comprised a mean of only 0.19% of total gastric lymph cells (0.25%, 0.06%, 0.18% and 0.25% for does 84, A1149, 20 and 27, respectively). At their peak response (mean 3.33%) these values were 5.64%, 2.95%, 2.92% and 1.82%, respectively.
Individual mean hourly output of IgA-containing cells is shown in Figure 5.17. Doe 84 exhibited a massive increase in IgA-containing cells on days 4 and 5 PI. A very much smaller response was recorded for doe 20 on days 6 and 7. IgA-containing cells accounted for 0.35% of total lymphocytes between days -1 and 1 in doe 84 and were almost completely absent from the other animals. At their maximum levels these cells made up just 4.04%, 0.8%, 2.91% and 0.8% of the total lymphocytes of does 84, A1149, 20 and 27, respectively (mean value 2.14%).

For clarity, the mean lymphocyte, lymphoblast and IgA-containing cell outputs obtained from the four does are shown in Figure 5. 18.
Figure 5.15  Mean hourly lymphocyte output following single challenge.

Figure 5.16  Mean hourly blast cell output following single challenge.
Figure 5.17 Mean hourly IgA-containing cell output following single challenge.

Figure 5.18 Mean hourly cell output following single challenge.
5.4 Discussion

The results of the field studies conducted over the two seasons demonstrate that it is possible, using the simplest parasitological parameter (faecal egg count), to segregate grazing adult female goats into responder and non-responder individuals. Whilst grazing, responsiveness to gastrointestinal nematode infection appeared to be a relatively stable characteristic unaffected by the presence of both gastric and intestinal nematodes.

The artificial challenge studies, however, provided evidence of seasonal effects upon responsiveness and in the case of the does challenged in 1995 some evidence that responsiveness may, for reasons that are not readily apparent, be a transient feature. The pattern of egg count seen in the *T. circumcincta* and *T. vitrinus* challenged responder does suggests that these animals lost their ability to regulate egg output from day 35 onwards, shortly after the termination of larval challenge. During the last three weeks of this study the egg counts of the responder and non-responder does were very similar. This finding was in marked contrast to those from the previous year’s doe study and the entire male study (Chapter 4) where significant differences in responsiveness were maintained throughout the period of observation. The worm burden data from the subsequent single challenge study conducted in 1995 also failed to provide evidence of any differences in responsiveness between these two groups. The quantitative and qualitative differences in worm burden between the two selected groups that were apparent in the first doe single challenge study conducted in 1994 were lacking in the second doe study. It is evident however, that the worm burdens of both groups killed in 1995 following a single challenge with 50,000 *T. circumcincta* were, in comparison with the previous year’s study,
relatively low: in the responders and non-responders killed in 1995, 11.7% and
12.7% of the larval challenge was recovered at slaughter compared to recoveries of
25.3% and 35.5% respectively in the does killed in the previous year.

The relatively low burdens in the 1995 study might be thought to provide
evidence of an equal expression of acquired immunity in both groups. However this
seems unlikely since the most obvious manifestation of acquired immunity seen in
the doe/ewe (Chapter 3), entire male (Chapter 4) and previous ovine studies (Seaton
et al., 1989a, b) was a marked retardation in the development of larval stages which
was not apparent in the 1995 doe single challenge study. The relatively low
recoveries in the 1995 single challenge study may have been attributable to some
reduction in larval viability or to some non-specific feature peculiar to that particular
study. Since the larvae used in both the 1994 and 1995 single challenge studies were
freshly cultured and had not been stored for more than three weeks it is difficult to
understand why there should be any difference in larval viability. However,
differences in establishment are not unprecedented for this strain of *T. circumcincta*;
in a study where this strain was radiolabelled, establishment in three groups of naive
challenge control lambs was variable ranging from 41.6, 40.3 to 24.0% (Seaton et
al., 1989b). The possibility that the relatively low worm populations seen in the 1995
doe single challenge study were a consequence of reduced larval viability which
affected larval establishment and/or persistence cannot be dismissed. Furthermore, in
anthelmintic disrupted challenge models the size of the population that becomes
established may also influence subsequent population regulation, since this
population provides the antigenic stimulus which is a vital component of effector
mechanisms. Antigenic thresholds have been proposed as a key component of the
effector mechanisms that regulate parasite populations since the earliest studies on immunoregulation of *Haemonchus* (Dineen 1963).

The acquisition and expression of immunity does not simply depend upon the antigenic exposure and genetic background of an individual but is influenced by a number of other interactive components. These include previous exposure to infection (Gill 1991), nutritional (Wagland et al., 1984) and reproductive status (O'Sullivan and Donald 1973) and animal management (Cabaret et al., 1989). For these reasons it is very difficult to determine exactly the aetiology of any changes that occur in the apparent susceptibility of animals during the course of an experiment. Since all of the does used in the studies reported here gave birth to and reared a single kid it is unlikely that reproductive/lactational differences could have accounted for the changes in susceptibility seen in the second single challenge study. However certain aspects of the management of the study may have contributed to the susceptibility of the herd. The removal from the breeding herd of the most and least susceptible does at the end of the first grazing season may, by reducing within herd variance in responsiveness, have served to restrict between group differences in the second year. The routine anthelmintic treatments given to the does not only made the interpretation of faecal egg count data difficult but it is possible that they may have also directly influenced the development and/or expression of immunity (Gibson et al., 1970; Luffau et al., 1990). The fact that the does grazed on an extensive hill pasture with a high sward species heterogeneity, coupled with the preferential grazing habits of goats, made it impossible to obtain pasture larval counts which might well have demonstrated seasonal differences in exposure to larval challenge. It is quite possible that there were indeed seasonal differences in parasite challenge and
nutrition that were directly attributable to differences in climate between the two 
grazing seasons. Marked differences were evident in the rainfall during the May-
October grazing seasons in 1993 and 1994. In the latter season rainfall levels 
between May and October were only 60% of what they had been the previous year 
(551 mm and 336 mm for 1993 and 1994, respectively).

The results from the grazing studies support the widely held view that faecal 
egg count is the simplest and most appropriate indicator of host resistance to 
gastrointestinal nematode infection, particularly when animals are required for 
subsequent breeding. Although FEC provides an accurate measure of total 
trichostrongylo (Bisset et al., 1991) and in particular current H. contortus burden 
(Roberts and Swan, 1981), it correlates less well with Teladorsagia spp. infection 
where egg output follows a markedly stereotypic pattern (Michel, 1969). 
Nevertheless, the use of FEC as an indicator trait is justified as regulation of egg 
production has a direct effect on subsequent pasture contamination (Albers and Gray, 
1986).

The ranking of individual egg counts over both grazing seasons enabled the 
identification of responder and non-responder does which exhibited significant 
differences in FEC both from each other and more importantly the herd mean. The 
significant values obtained for repeatability of herd egg count within each season and 
the high estimates of repeatability between FEC on pasture and following artificial 
continuous challenge provide support for the use of FEC rankings as an indication of 
host resistance in does. The non-significant repeatability estimates calculated for the 
selected responders and non-responders in both years may well be a function of 
small group size, low individual egg counts, the high degree of variation present in
FECs and the anthelmintic disrupted challenge model used in this study. Ovine selection studies conducted in New Zealand have used sheep naturally infected with Teladorsagia spp. and Trichostrongylus spp. and have suggested that egg counts arising from reinfection post-treatment may be most informative as they reflect the ability of the host to regulate larval establishment and development (Baker et al., 1990). Previous selection studies in Australia and New Zealand have also utilised FEC though selection regimes differ. The Australian studies have relied heavily on artificial infection; a single artificial challenge superimposed on naturally acquired H. contortus infection, or vaccination and homologous challenge in the T. colubriformis lines (Windon, 1990). Studies in New Zealand have followed the responses of grazing sheep to naturally acquired infection (Baker et al., 1990).

The results of these selection studies show the resistance of female goats to nematode infection to be a relatively constant characteristic, with animals occupying similar positions of responsiveness on pasture and when housed and artificially infected. Although the FEC pattern following artificial infection varied between years, the responses to challenge infection supported selection on pasture with responders shedding significantly fewer eggs than non-responder and unselected does. The stability of individual responsiveness is illustrated by the significant FEC repeatability estimates obtained on pasture (0.19 and 0.26 in 1992-93 and 1993-94, respectively) and in particular following artificial challenge (0.70, 0.70 and 0.74 for responder, non-responder and unselected does in 1994, and 0.83 and 0.45 for responders and non-responders in 1995), and the high correlations calculated between mean FEC on pasture and post-challenge (0.78 and 0.73 in 1994 and 1995, respectively). These estimates compare favourably with those obtained from
previous sheep studies. Barger and Dash (1987) reported FEC repeatability estimates of approximately 0.6 in young Merino wethers which were housed and given a trickle challenge with *H. contortus*. Studies using female Scottish Blackface lambs given two single infections with 50,000 *T. circumcincta* separated by anthelmintic treatment estimated the repeatability of FEC between infections to be 0.3 and the repeatability for successive samples, taken at 2-3 day intervals, to be 0.75 (Stear, Bishop, Duncan, McKellar and Murray, 1995). The current estimates are similar to those obtained in the previous buck selection studies (Chapter 4), but differ from those reported for goats in the tropics (Woolaston *et al.*, 1992b) where the authors recorded very low FEC repeatabilities in animals infected with *T. colubriformis* and *H. contortus* in the early years of the study and with *T. colubriformis* alone in the later years. However it is possible that these differences may be influenced by differences in grazing management, nutrition and nematode parasitism between tropical and temperate environments. Though repeatability estimates may be influenced by both genetic and environmental factors, they indicate the limit of the trait’s genetic determination and heritability (Falconer, 1981).

Examination of the egg count data gathered from the entire doe herd provided evidence of consistent patterns of seasonal prevalence of the principal parasites on pasture at Sourhope and of seasonal changes in faecal consistency. Specific egg counts conducted on faecal samples collected from grazing does during the course of the study show a succession of species. Both *T. circumcincta* and *T. vitrinus* eggs feature in the post-partum egg counts of the does, but the former species predominates during the summer months. Over the late autumn and winter
months *T. vitrinus* eggs predominate in doe faeces, at which time the majority of the *T. circumcincta* infrapopulation is present in a state of hypobiosis.

However it was very apparent that there was a marked post-parturient increase in faecal egg count in non-responders that was not apparent in the herd as a whole and did not occur in the responder does. Breeding ewes are well known to exhibit an increase in FEC around the time of pregnancy and lactation. This periparturient relaxation in immunity has been observed in a number of laboratory studies and has recently been recorded in goats (Rahman and Collins, 1992). The relaxation in immunity may be a result of the stresses of lactation as the increased susceptibility of the dam is abolished by removal of the suckling lamb (O’Sullivan and Donald, 1973). The hormone prolactin has been implicated in the PPR (Barger, 1993a) though recent ovine studies have shown that although prolactin may have a role in maintaining the response it does not in itself initiate it (Coop et al., 1990; Jeffcoate et al., 1990). Several ovine studies have suggested that dam susceptibility may be species-specific. O’Sullivan and Donald (1973) reported increased susceptibility towards *T. colubriformis* but not *H. contortus* infection, while in a Scottish study Jackson et al. (1988a) recorded an increased ewe susceptibility pre-partum to *T. circumcincta* but not *T. vitrinus*.

Overdispersion is a common phenomenon in many parasite systems (Barger, 1985) and the results of this study suggest that overdispersion may also play an important role in nematodiasis in fibre goats. The contribution to pasture contamination made by the most susceptible animals during the months of May-September when conditions favour larval development and transmission was about twice that made by the average doe and 4 times that made by the least susceptible
animals. These figures illustrate the potential benefits of enhancing herd responsiveness either through the selection of the most or removal of the least responsive animals.

There was a noticeable reduction in faecal consistency of all the does during the two months following turnout in May. Faeces became looser and unformed in all does over that period but these changes were reversed towards the end of the grazing season. Although it has been suggested that faecal consistency may reflect the level of larval challenge (Larsen et al., 1994; Suttle and Brebner, 1995) this change may also be associated with the dietary/nutritional changes that occur when does move back onto pasture. Studies with sheep in New Zealand have described a relationship between the prevalence of fleece dags, due to more liquid faeces, and host responsiveness and have suggested that selection for reduced FEC may lead to increased dag prevalence (Watson et al., 1986). The results of the doe study show no differences between the faecal consistency of responders and non-responders and no evidence of diarrhoea. The diarrhoea seen in sheep studies may be attributable to the greater responsiveness of that species to gastrointestinal nematodes.

These studies provide the first information on sex-related differences in responsiveness between breeding male and female goats. Although a direct comparison with the bucks in the previous study (Chapter 4) is not strictly valid due to differences in ages, times of slaughter and previous exposure to infection, the results suggest that female goats are more resistant to gastrointestinal nematode infection. Though there was a high degree of variation in FEC within the selected groups of either sex after trickle challenge, mean female responder counts were lower than those of responder bucks and male non-responders had a mean egg count
almost twice that of non-responder does. Indeed, mean counts in unselected does were similar to those of selected responder males. The increased susceptibility of intact males to nematodiasis has been reported in a number of laboratory and field studies though the reasons for it remain unclear (Barger, 1993a).

Worm burden analysis 10 days after challenge with 50,000 *T. circumcincta* L₃ illustrated differences in both total numbers and stage composition of responder and non-responder populations though these were complicated by between-year variation. In the first year of the study mean responder burden was approximately three-quarters that of non-responder and unselected does though in the second year there was only a 10% difference. There was also considerable variation in the rates of larval development between groups and years. In responders in the first year the majority of the established larvae were still at the early and mid L₄ stages while a much greater proportion of late L₄ and fifth-stage worms were recovered from non-responder and unselected animals. The results from the second year showed few differences in the proportion of larval stages recovered from responders and non-responders. Previous studies with sheep have shown that by day 10 post-infection the majority of *T. circumcincta* larvae will have reached the early adult or fifth-stage (Denham, 1969). Ovine acquired resistance to *T. circumcincta* is first expressed through a reduction in the rate of development of established larval stages (Seaton *et al.*, 1989b). The results of the first year’s study and the previous ewe/doe study (Chapter 3) suggest that effects upon larval development are an important feature of caprine acquired immunity to gastrointestinal nematode infection. Responsive individuals appear to be able to regulate their nematode burdens through a reduction in the rate of larval development.
Although it is accepted that local cell mediated responses are crucial components of effector mechanisms that regulate gastrointestinal nematode infections (Miller 1984; Rothwell 1989), peripheral responses, particularly those that can be measured in blood, have been the subject of considerable study as these may have some predictive value. The results from the chicken albumin study, in which there were no differences in the peripheral responses of responder and non-responder does, suggest that the ability to regulate nematode populations is not linked to the ability to mount effective circulating antibody responses.

The proliferation of peripheral and tissue eosinophils has been closely associated with nematode infection (Rothwell, 1989; Jones, 1993) and may play an important role in the host inflammatory response (Rothwell and Dineen, 1972). Though there were differences in blood eosinophil levels between years it is clear from these studies that peripheral eosinophil numbers increased significantly faster in responders than in non-responders to reach significantly higher levels within three weeks of first infection. Non-responders, on the other hand, mounted a slower response that was generally of a lesser magnitude. The finding that responders had a significantly faster and more pronounced blood eosinophil response than non-responders is in agreement with the results from similarly challenged entire male goats (Chapter 4) and concurs with ovine studies which have suggested that high peripheral eosinophil levels following challenge may reflect host responsiveness rather than the level of infection (Dawkins et al., 1989). A number of laboratory studies have suggested that the ability to mount a rapid and vigorous eosinophil response may be an important determinant in host resistance (Handlinger and Rothwell, 1981; Lawrence and Pritchard, 1994). Although there was considerable
variation in abomasal tissue eosinophil counts between the two years of the study, mean responder counts were consistently greater than those of non-responders. The high numbers of tissue eosinophils present in responders accords with their positive relationship with resistance reported from a number of ovine studies (Dineen and Windon, 1980; Douch et al., 1986; Gill, 1991). A positive relationship (Dineen et al., 1978) and absence of any association (Gregg et al., 1978) between intestinal eosinophil numbers and T. colubriformis burden has also been described. The absence of any consistent relationship between tissue eosinophil level and worm numbers within either responders or non-responders in the current study may be largely due to the small number of animals involved.

Mucosal mast cell, and in particular globule leukocyte, proliferation is a well documented response of ruminants to helminthiasis (Miller, 1984; Rothwell, 1989) which may be associated with the development and expression of acquired resistance to nematode infection (Askenase, 1980; O’Sullivan and Donald, 1973; Huntley et al., 1984). The results of the present studies provide a degree of support for these findings as there was some evidence for increased MMC and GL numbers to be associated with lower day 10 worm burdens and a retardation in larval development. Interestingly, though MMC and GL numbers varied between groups and over years, the proportion of mast cells present as GLs was very constant. Since the proportion of GLs was very similar for all groups studied and the does in the second year had fewer MMCs and GLs with lower worm burdens than the previous year, it is most likely that any role for mast cells in host resistance is qualitative rather than quantitative. This is in agreement with the results of Huntley et al. (1992) who suggested that the presence of mast cells was not a pre-requisite for immunological
effector function. The MMC and GL levels reported in the present studies are very much greater than those recovered from randomly selected does in a previous study (Chapter 3). The values obtained in the second year are similar to those described earlier for selected responder bucks (Chapter 4). However, as the animals used in these studies varied in their previous parasite exposure and treatment and infection regimes a direct comparison is not possible. There were no significant differences in SMCP concentrations between groups in either year, and, although there was limited evidence to suggest that SMCP levels may have been associated with increased resistance, this was very weak. As previous studies have demonstrated that maximal SMCP concentrations in immune sheep are to be found 6-8 days after challenge with *T. colubriformis* (Bendixsen *et al.*, 1995), the apparent absence of any relationship between SMCP concentration and worm burden in the present study suggests that any influence SMCP has on the immunoregulatory process is expressed prior to day 10 post-challenge.

The lymphatic cannulation study was intended to provide preliminary data regarding the local immune responses at the site of infection. However although this study had limited success in terms of the numbers of animals from which gastric lymph was obtained, it did provide some interesting results. Though a similar proportion of goats were successfully cannulated as has been achieved for sheep undergoing an identical procedure (F. Jackson, personal communication) a very large proportion of the does (86%) stopped flowing within 48 hours of surgery. This is most likely to have been due to the low lymph flow rates recorded for the does compared to previous ovine studies (Smith *et al.*, 1981). Though there are not sufficient results to enable comparison between responsive and non-responsive goats.
the results can be compared with those reported from a similar study using sheep resistant to *T. circumcincta* infection (Smith *et al.*, 1984a). Though there was considerable variation between individuals there was evidence of an increase in total cell output from day 2 PI to a maximum on days 4-5. A similar cellular response has been reported previously (Smith *et al.*, 1983a, b) and may be influenced by increased voluntary feed intake. The cellular responses were most pronounced in the previously selected responder doe with much lower responses detected in the other animals. The timing of the responses also varied between individuals. Though the maximum lymphoblast response in the selected responder was on day 4, peak response occurred on day 5 for two of the does (A1149 and 27) and not until day 9 for one non-responder (doe 20). Similarly, peak IgA-containing cell output was on days 4-5 for the responder doe but not until days 6-7 for the non-responder doe 20. Though very low, maximum IgA-containing cell output in the other two animals was between days 5-6 PI. Comparison of the cellular responses of the four does suggested that the responses of the selected responder doe occurred earlier and were of a greater magnitude than those of the more susceptible animals. It is possible that, as with the eosinophil response (Handlinger and Rothwell, 1981; Lawrence and Pritchard, 1994), the ability to mount a more dynamic local cellular response is an important factor in deciding host resistance.

These results make interesting comparison with those obtained following a similar challenge infection administered to resistant sheep (Smith *et al.*, 1983b, 1984a) where a marked cellular response was detected over days 2 to 5 post-challenge. The most obvious difference is in the magnitude of the cellular responses. Hourly lymphocyte, lymphoblast and IgA-containing cell output was always
considerably lower in the goats than has been recorded for sheep (Smith et al., 1983b, 1984a). Though these differences were not as extreme they were still apparent for the selected responder doe. Nevertheless, although the does exhibited much lower peak cellular responses the results show that as they started with very low cell output rates, the increases seen in the post-infection responses were similar in degree to those of ewes given an identical challenge (Smith et al., 1983b).

Previous ovine studies have suggested that as the majority of *T. circumcincta* larvae establish within two days of infection (Armour et al., 1966) the cellular response occurs too late to prevent larval establishment. However this response may be involved in the cell-mediated mechanisms responsible for retardation in larval development and subsequent worm expulsion (Smith et al., 1983b, 1984a). The importance of IgA in the immune responses of sheep infected with *T. circumcincta* is unclear as Smith et al. (1983a) reported an increase in production of IgA-containing cells and immunoglobulin in the gastric lymph of lactating ewes which were more susceptible to *T. circumcincta* challenge.

The results of the field and housed studies described here demonstrate that Scottish cashmere does can be successfully segregated into responsive and non-responsive individuals using simple parasitological criteria. These studies illustrate differences in susceptibility between breeding male and female goats to nematode infection and provided the first data relating to differences in the local immune responses of ewes and does to *T. circumcincta* infection.
Chapter 6 - Responses of helminth-line animals
Selective breeding programmes to enhance ruminant responsiveness to nematodiasis can only be economically viable if resistance to nematode infection is significantly heritable and selection does not have unacceptable adverse results upon productivity. Published estimates for the heritability of ovine resistance to nematode infection, determined using FEC, are moderate ranging from 0.23 (Piper, 1987) to 0.44 (Woolaston et al., 1991). These values are similar in magnitude to those calculated for production traits such as fleece and body weights. However the only published estimates for the heritability of resistance in goats were not significantly better than zero (Woolaston et al., 1992b). The earliest ovine studies suggested that selection for increased resistance should have very little if any effect on productivity (Albers et al., 1987; Woolaston, 1990). Albers et al. (1987) have shown that the relationship between egg count and productivity is close to zero in unchallenged environments but becomes favourable under conditions of parasite infection. More recently, studies in New Zealand have reported an enhanced susceptibility to nematodoses in Romney sheep selected for increased fleeceweight (Howse et al., 1992; Williamson et al., 1994) and in other studies from the same country enhanced responsiveness has been associated with increased soiling of the fleece around the breech and consequently some reduction in wool production (Watson et al., 1986; Baker et al., 1991).

Previous studies have suggested that there may be important differences in drug pharmacokinetics between sheep and goats with the result that anthelmintic treatments are less efficacious in goats (Sangster et al., 1991; Charles et al., 1989). Ali and Hennessy (1993) have suggested that reducing feed intake prior to
administration of anthelmintic may lead to an increased drug bioavailability as a result of reduced digesta flow. As the animals were available, a faecal egg count reduction test (FECRT) was conducted to determine if there were any differences in the efficacy of ivermectin and levamisole when given to helminth-line or unselected-line male or female kids, and whether these differences could be reduced by prior fasting of the animals.

The aims of the present studies were to compare the FEC responses and productivity of selected helminth-line and unselected fibre-line male and female kids and yearlings following artificial and natural challenge infection. These studies should provide preliminary information on the heritability of resistance in goats and show if any deleterious effects upon growth and cashmere fibre production were evident in the first two generations of selected line kids. The observations made on the entire male kids in the selected line would also be used to select the next generations of 'helminth-line' sires.

6.2 Material and Methods

6.2.1 Animals

Kids were born between late April and early June of each year. They remained on pasture alongside their dams from birth until weaned which was at 12 weeks of age for the males and 16 weeks for females. Following weaning the kids were grazed in single sex groups together with an equivalent number of fibre-line kids prior to housing in October/November of their first year. Housed kids were accommodated in straw bedded paddocks and maintained on a diet of 'Green Keil'
(Central Farmers Ltd., 16% crude protein) and hay and water \textit{ad libitum}. The goats were turned out onto pasture as yearlings in early May.

\subsection*{6.2.2 Herd management}

These studies were conducted using helminth-line kids born in 1993, 1994 and 1995 (see Table 6.1). The sires of the kids born in 1993 and 1994 were those responder bucks described in Chapter 4. Thus the kids born in the first two years of the study are first generation animals. From autumn 1994 breeding bucks were selected from among the males born into the helminth-line (HL) the previous year. The kids born in 1995 are thus second generation animals. The male and female kids used to provide control data, the unselected fibre-line (UFL) kids, came from an unselected line of kids that are used to provide control data for the selected fibre lines being developed by MLURI and the Roslin Institute, Edinburgh, in conjunction with the Scottish Cashmere Producers Association.

\begin{table}[h]
\centering
\caption{Outline of background of helminth-line kids.}
\begin{tabular}{lll}
\hline
Kids & Sires & Dams \\
\hline
1993 first-generation. & F8002 and C9053 selected from initial 83 bucks. & Initial breeding herd of 95 does. \\
1994 first-generation. & F8002, D1195 and H8088, selected from initial 83 bucks. & Second season’s breeding herd of 95 does. \\
1995 second-generation. & Top 2 responders selected from males born into helminth-line in 1993. & Third season’s breeding herd of 95 does including 30 females born into helminth-line in 1993. \\
\hline
\end{tabular}
\end{table}
6.2.3 Responses to artificial and natural infection

1993 Kids

In October of 1993 the HL kids were housed together with a similar number of UFL kids. All animals were treated with anthelmintic (ivermectin, 400 \( \mu \text{g kg}^{-1} \)) on 10 consecutive days beginning on March 28\(^{\text{th}}\) 1994. Three weeks post-treatment the males were artificially infected with 10,000 *T. circumcincta* L\(_3\). Faecal egg counts and peripheral eosinophil counts were conducted fortnightly following infection. On May 9\(^{\text{th}}\) 1994 all the yearlings were turned out to graze on contaminated pasture. Rectal faecal samples were collected from the females for egg count analysis every 5 weeks immediately prior to anthelmintic treatment (levamisole, 12 mg kg\(^{-1}\)). In each year 6 responder and 6 non-responder males and females were identified from the HL on the basis of their post-challenge egg counts as described previously (Chapter 4). The top responder male yearlings were kept aside for use in the selective breeding programme (Chapter 4).

1994 Kids

The second crop of first generation kids, born in 1994, were housed on November 10\(^{\text{th}}\) 1994 with an equal number of UFL kids, treated with ivermectin (400 \( \mu \text{g kg}^{-1} \) on 10 consecutive days) and on January 25\(^{\text{th}}\) 1995 challenged with 10,000 *T. circumcincta* L\(_3\). Egg counts and blood eosinophil counts were conducted for all animals every 2 weeks post-infection. The yearlings were turned out onto contaminated pasture on May 9\(^{\text{th}}\) 1995. The 6 most and 6 least responsive male and female HL yearlings were identified and the top responder males kept as breeding stock for the next season (see Chapter 4).
The second generation kids born in 1995 were followed on pasture between late July and housing on November 8th 1995. A faecal egg count reduction test (FECRT) was conducted at housing using the HL kids and an equivalent number of UFL kids in order to test differences in anthelmintic efficacy. The animals were randomly divided into 4 treatment groups each containing an equal number of males and females from each of the 2 lines. Treatment groups were balanced as far as possible in terms of weight and previous FEC. At housing (day 0) the kids were faecal sampled and randomly assigned to one of four drenching regimes; ivermectin (Oramec, 200 μg kg⁻¹), levamisole (Levacide, 7.5 mg kg⁻¹), or starved for 24 hours prior to either ivermectin or levamisole treatment. Further egg counts were conducted on days 9 and 21. The efficacy of each drug treatment was calculated by dividing the difference between pre- and post-treatment FECs by egg count on day 0. During the course of the FECRT the kids were not exposed to reinfection.

6.2.4 Artificial challenge of first generation bucks

In the summer of 1995 the 5 top responder bucks which had been born in 1993 and kept for breeding the previous autumn were housed at Moredun together with 5 similarly aged breeding males from the Sourhope fibre-line. These animals were housed in a single group, treated with ivermectin (400 μg kg⁻¹ on 10 consecutive days) and maintained on a diet of ESCA nuts (14% crude protein) and hay and water ad libitum. Twenty-one days after the last anthelmintic treatment the bucks were given a mixed trickle challenge with 2000 *T. circumcincta* and 1000 *T. vitrinus* L₃ per day, 5 days per week for 4 weeks as previously described (Chapter 4). Faecal egg counts were conducted twice weekly and peripheral eosinophil numbers
calculated weekly after first infection. On day 60 after first infection the bucks were treated with anthelmintic (Panacur, 10 mg kg\(^{-1}\)) and on day 67 given a single oral challenge with 50,000 *T. circumcincta* L\(_3\). Ten days later, day 77, the bucks were killed and their worm burdens calculated as described previously (Chapter 2).

6.2.5 Eosinophil counts

Venous blood was drawn into an EDTA-containing vacutainer tube and peripheral eosinophil counts conducted following fixing with Carpentier's stain (Chapter 2).

6.2.6 Post-mortem techniques

The abomasum of each animal was removed, and the numbers and stage of development of the worms recovered from 2% aliquots determined. Immediately after slaughter excised sections of abomasal folds were processed for the enumeration of mast cells, globule leukocytes, tissue eosinophils and SMCP concentrations as described in Chapter 2.

6.2.7 Statistical analysis

Statistical analyses were conducted using the untransformed mean data for each individual and the effect of group tested using the Mann-Whitney test. Estimates of repeatability of FEC were obtained by calculating the degree of correlation between adjacent log\(_{10}(x + 1)\) transformed samples within each group. FEC repeatability estimates were analysed using the 1-sample Wilcoxon test.

Heritability estimates for enhanced resistance to gastrointestinal nematode infection were calculated using average egg counts obtained for the bucks and does in 1992, male and female HL and UFL yearlings in 1994 and male HL and UFL yearlings in 1995 (see Chapter 2).
6.3 Results

6.3.1 Responses to artificial and natural infection

1993 Kids

The mean (± SEM) egg counts of HL and UFL male yearlings following artificial and natural pasture infection in 1994 are shown in Figure 6.1. Mean UFL egg count (170 ± 12 epg) was slightly lower than that of HL males (194 ± 15 epg) though these differences were not statistically significant. FEC repeatability estimates calculated for the HL line male kids between their first and second season were very low (r = -0.12). Egg count analysis following artificial and natural challenge showed no evidence of any sire-related differences in FEC among HL males.

Within the yearling males of the HL it was possible to identify individuals which were consistently occupying positions of high or low responsiveness. Through the retrospective analysis of FECs following artificial and natural infection 6 responder and 6 non-responder animals were identified (Figure 6.2). Following artificial and natural challenge selected responder egg count (112 ± 16 epg) was significantly lower than that of selected non-responders (301 ± 30 epg, p<0.01) and the mean of the HL males (p<0.01). Non-responder FEC was significantly greater than the mean of the HL yearlings (p<0.01).

The mean (± SEM) egg counts of HL and UFL female yearlings resulting from naturally acquired reinfection following anthelmintic treatment on pasture in 1994 are shown in Figure 6.3.
Figure 6.1. Mean (± SEM) FECs of HL and UFL male yearlings, 1994.

Figure 6.2. Mean (± SEM) FECs of 6 responders and 6 non-responders selected from the HL yearling males, 1994.
Although there was no significant difference between the overall mean HL and UFL FECs, HL females had significantly lower counts in June (p<0.05) and August (p<0.01). There was no apparent correlation between mean FEC on pasture in the first and second season for HL females (r = 0.17). Over the period of natural reinfection on pasture females sired by F8002 had significantly lower egg counts than did females sired by C9053 (91 ± 11 epg and 114 ± 11 epg, respectively, p<0.05). Though the mean egg count of the 6 HL responder females was lower than that of non-responders (30 ± 7 epg and 103 ± 39 epg, respectively) this was not significant (Figure 6.4).

1994 Kids

The mean (± SEM) FECs of the HL and UFL males from the second kid crop following artificial and natural challenge are shown in Figure 6.5.

At the end of the field study mean HL egg count was significantly less than that of the UFL yearlings (159 ± 6 epg and 249 ± 14 epg, respectively, p<0.001). The estimate of repeatability of mean FEC for HL males between the first and second season was 0.34. There was no evidence of any sire-related effect on egg count. The FECs of the 6 selected responder and 6 selected non-responder yearlings are shown in Figure 6.6. Over the course of the study mean responder FEC was significantly lower than that of the non-responders (117 ± 7 epg and 211± 5 epg, respectively, p<0.005). Responder and non-responder FECs were significantly different from the mean for the HL (p<0.005).
Figure 6.3. Mean (± SEM) FECs of HL and UFL female yearlings, 1994.

Figure 6.4. Mean (± SEM) FECs counts of 6 responders and 6 non-responders selected from the HL yearling females, 1994.
Figure 6.5. Mean (± SEM) FECs of HL and UFL yearling males, 1995.

Figure 6.6. Mean (± SEM) FECs of 6 responders and 6 non-responders selected from the HL yearling males, 1995.
In the second year, there were no significant differences between the HL and UFL females in mean egg count following artificial and natural challenge (Figure 6.7). Faecal egg counts in the first and second season were moderately repeatable for the HL females \((r = 0.32)\). There were no differences between sire groups in mean egg count of yearling HL females. Figure 6.8 shows the FECs of the selected 6 responders and 6 non-responders following artificial and natural infection. Selected HL responders had significantly lower mean egg counts than did the non-responders \((64 \pm 6 \text{ and } 214 \pm 9 \text{ epg, respectively}, \ p<0.005)\). Mean responder and non-responder counts were significantly different from the group mean of 128 \((\pm 7)\) epg \((p<0.001)\).

**Faecal consistency scores**

Male HL and UFL yearling faecal consistency score fell significantly \((p<0.001)\) immediately after turnout in May 1995 (Figure 6.9). Faecal consistency score dropped dramatically in both HL and UFL females immediately post-turnout (Figure 6.10). There were no differences in consistency score between groups.

**6.3.2 Artificial challenge of first generation bucks**

Mean \((\pm \text{ SEM})\) FECs of the 5 HL and 5 UFL bucks following artificial trickle challenge in 1995 are shown in Figure 6.11. Between days 18-60 after first infection the mean egg count of the HL responders was significantly lower than that of the UFL bucks \((345 \pm 41 \text{ epg compared to } 559 \pm 58 \text{ epg, respectively}, \ p<0.05)\).
Figure 6.7. Mean (± SEM) FECs of HL and UFL female yearlings, 1995.

Figure 6.8. Mean (± SEM) FECs of 6 responders and 6 non-responders selected from the HL yearling females, 1995.
4.00
Faecal Scores

Mar Apr May Jun Jul Aug Sep
Month

3.00

Figure 6.9. Mean (± SEM) faecal consistency scores of male yearlings, 1995.

4.00
Faecal Score

Feb Mar Apr May Jun Jul Aug Sep
Month

3.00

Figure 6.10. Mean (± SEM) faecal consistency scores of female yearlings, 1995.
6.3.3 Worm burden analysis

Day 10 *T. circumcincta* burdens recovered from the HL and UFL breeding-bucks are shown in Figure 6.12. The mean worm burden of the breeding HL bucks was $16,375 \pm 1330$ (Table 6.2). This was not significantly different from that of the UFL bucks ($13,310 \pm 1778$). There were no significant differences in worm burden composition between groups (Table 6.3). There was no association between mean FEC following artificial trickle infection and *T. circumcincta* numbers 10 days post-challenge.

**Table 6.2. Mean (± SEM) day 10 *T. circumcincta* burdens, 1995.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Early L₄</th>
<th>Mid L₄</th>
<th>Late L₄</th>
<th>Stage 5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL</td>
<td>1334</td>
<td>7545</td>
<td>6511</td>
<td>985</td>
<td>16,375</td>
</tr>
<tr>
<td></td>
<td>(± 203)</td>
<td>(± 1583)</td>
<td>(± 1363)</td>
<td>(± 470)</td>
<td>(± 1330)</td>
</tr>
<tr>
<td>UFL</td>
<td>1210</td>
<td>7744</td>
<td>3991</td>
<td>365</td>
<td>13,310</td>
</tr>
<tr>
<td></td>
<td>(± 345)</td>
<td>(± 1244)</td>
<td>(± 979)</td>
<td>(± 63)</td>
<td>(± 1758)</td>
</tr>
</tbody>
</table>

**Table 6.3. Mean (± SEM) stage composition of *T. circumcincta* burdens, 1995.**

<table>
<thead>
<tr>
<th>Group</th>
<th>% of established worms recovered as...</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early L₄</td>
</tr>
<tr>
<td>HL</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>(± 1.9)</td>
</tr>
<tr>
<td>UFL</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td>(± 3.3)</td>
</tr>
</tbody>
</table>
Figure 6.11. Mean (± SEM) FECs of HL and UFL bucks following artificial trickle challenge, 1995.

Figure 6.12. Individual *T. circumcincta* burdens, 1995.
6.3.4 Eosinophil counts

1993 Kids

Mean (± SEM) peripheral eosinophil counts of the HL and UFL male yearlings following artificial and natural challenge in 1994 are shown in Figure 6.13. There were no differences between groups in either the rate or magnitude of the eosinophil response. Within each group blood eosinophil numbers increased dramatically immediately after turnout reaching peak levels approximately 5-9 weeks post-turnout. However there was evidence of non-significant differences in the eosinophil responses of the selected responder and non-responder HL yearlings from the (Figure 6.14). Though responders and non-responders had almost identical eosinophil levels on day 0, eosinophil numbers increased noticeably faster in responders. The non-responder response was of a similar magnitude but was approximately 2-3 weeks later in developing.

1994 Kids

The peripheral eosinophil responses of the HL and UFL male yearlings challenged in 1995 are shown in Figure 6.15. There was little difference in the responses of the HL and UFL males with both groups showing an increase in eosinophil numbers at turnout and again in July. There was a very pronounced response in late August which may be connected with increasing FECs at this time. There were no differences in blood eosinophil levels of selected responder and non-responder male HL yearlings (Figure 6.16).
Figure 6.13. Mean (± SEM) peripheral eosinophil counts of HL and UFL male yearlings, 1994.

Figure 6.14. Mean (± SEM) peripheral eosinophil counts of 6 responders and 6 non-responders selected from the HL yearling males, 1994.
Mean (± SEM) female circulating eosinophil levels in 1995 are shown in Figure 6.17. There were no differences in eosinophil levels between the 2 groups after artificial and natural infection. Peripheral eosinophil numbers remained very low following turnout with the first signs of a response not occurring until July.

**Eosinophil counts of first generation bucks post-challenge, 1995**

The mean (± SEM) peripheral eosinophil counts of the 5 HL responder and 5 UFL breeding bucks following trickle challenge in 1995 are shown in Figure 6.18. Circulating eosinophil numbers increased significantly by day 21 (p<0.05) reaching peak levels on day 28 after first infection. Maximum HL responder eosinophil response was significantly greater than that of the UFL bucks (p<0.05). There was little change in blood eosinophil levels in the UFL animals. There were no differences in circulating eosinophil numbers between the two lines following single *T. circumcincta* infection.

There were no significant differences in abomasal eosinophil numbers recovered from HL and UFL bucks on day 10 post-challenge (Table 6.4). There was no relationship between tissue eosinophil numbers and worm burden or composition.

**6.3.5 Mast cell response**

HL and UFL bucks had very similar day 10 MMC and GL numbers (Table 6.4). There was no association between MMC or GL number and worm burden or composition. The proportion of mast cells present as GLs was almost identical in the two groups. Day 10 SMCP concentrations were not significantly different between HL and UFL bucks (Table 6.4).
Figure 6.15. Mean (± SEM) peripheral eosinophil counts of HL and UFL male yearlings, 1995.

Figure 6.16. Mean (± SEM) peripheral eosinophil counts of 6 responders and 6 non-responders selected from the HL yearling males, 1995.
Figure 6.17. Mean (± SEM) peripheral eosinophil counts of HL and UFL female yearlings, 1995.

Figure 6.18. Mean (± SEM) peripheral eosinophil levels of HL and UFL bucks following artificial trickle challenge, 1995.
Table 6.4. Mean (= SEM) mucosal mast cell (MMC), globule leukocyte (GL), % of
MMC as GLs (% GL), tissue eosinophil (Eos) and SMCP values for bucks, 1995.

<table>
<thead>
<tr>
<th>Group</th>
<th>MMC</th>
<th>GL</th>
<th>% GL</th>
<th>Eos</th>
<th>SMCP (µg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL</td>
<td>25</td>
<td>10</td>
<td>29</td>
<td>3.4</td>
<td>1.89</td>
</tr>
<tr>
<td></td>
<td>(± 3)</td>
<td>(± 2)</td>
<td>(± 1.0)</td>
<td></td>
<td>(± 0.34)</td>
</tr>
<tr>
<td>UFL</td>
<td>18</td>
<td>10</td>
<td>35</td>
<td>2.2</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>(± 3)</td>
<td>(± 4)</td>
<td>(± 1.1)</td>
<td></td>
<td>(± 0.31)</td>
</tr>
</tbody>
</table>

6.3.6 Production parameters

1993 Kids

There were no significant differences in body weight or weight gain between
the HL and UFL yearlings. Analysis of the production records (Table 6.5) indicated
that there were no significant differences between the HL and UFL in either the
mean quantity (240 ± 15 g and 247 ± 16 g, respectively) or economic value (£5.82
and £6.14, respectively) of the fibre produced by the male yearlings in 1994. In
contrast, in 1994 mean fibre production by female HL yearlings was significantly
greater than those of the UFL (291 ± 15 g and 242 ± 8 g, respectively, p<0.05). The
mean value of the cashmere obtained from females of the HL was significantly
higher than that of UFL females (£7.02 and £5.74, respectively, p<0.05).

Although the selected HL responder males were slightly heavier than the non-
responders this difference was at no point significant. Mean responder fibre
production and value (255 g and £6.00, respectively) were greater than those of
selected non-responders (210 g and £5.20, respectively) though these differences
were not significant. There were no significant differences between selected
responder and non-responder females in body weight, fibre production (292 g and
306 g, respectively) or fibre value (£7.40 and £7.00, respectively).

1994 Kids

There were no significant differences in weight gain or body weight between
male and female HL and UFL yearlings. Mean fibre production and value were not
significantly different between male HL and UFL yearlings (Table 6.5). Males of the
HL produced a mean of 263 (±15) g of fibre valued at approximately £7.28. The
equivalent figures for the UFL males were 276 (±13) g at £7.40. Helminth-line
female yearlings produced a mean of 219 (±9) g of fibre worth £5.62 (Table 6.5).
Mean output and value were both significantly lower than those obtained for UFL
females (289 ± 12 g and £7.65, respectively, p<0.001).

Selected HL responder males in the second year were not significantly
heavier than non-responders. Mean fibre production and value were higher in non-
responders (296 g and £7.80, respectively) compared to responders (231 g and £6.30,
respectively). These differences were not significant. There were no differences in
female responder and non-responder body weights. Selected HL female responders
produced more fibre (230 g) with a greater mean value (£5.20) than did non-
responders (173 g and £4.60, respectively).
Table 6.5. Mean (= SEM) cashmere fibre production from 1993 and 1994 kids.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th></th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HL</td>
<td>UFL</td>
<td>HLR</td>
</tr>
<tr>
<td>1993 kids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (g)</td>
<td>240</td>
<td>247</td>
<td>255</td>
</tr>
<tr>
<td>(± 15)</td>
<td>(± 16)</td>
<td></td>
<td>(± 15)</td>
</tr>
<tr>
<td>Value</td>
<td>£5.82</td>
<td>£6.14</td>
<td>£6.00</td>
</tr>
<tr>
<td>1994 kids</td>
<td>263</td>
<td>276</td>
<td>296</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>(± 15)</td>
<td>(± 13)</td>
<td>(± 9)</td>
</tr>
<tr>
<td>Value</td>
<td>£7.28</td>
<td>£7.40</td>
<td>£7.80</td>
</tr>
</tbody>
</table>

*figures with different superscripts are significantly different (p<0.05)*

HL = helminth-line, UFL = unselected fibre-line
HLR = helminth-line responders, HLNR = helminth-line non-responders

6.3.7 Faecal egg count reduction test

The mean percentage reduction in FEC at days 9 and 21 post-treatment for HL and UFL male and female kids are shown in Table 6.6.

There was evidence of considerable differences in drug efficacy, ivermectin being consistently more effective at controlling egg production than was levamisole, and anthelmintic treatments being considerably more efficacious in females than males. Starving for 24 hours prior to drenching did not appear to exert much influence on efficacy as measured on day 9 and 21 post-treatment.
Table 6.6. Percentage reduction in FEC at days 9 and 21 after anthelmintic.

<table>
<thead>
<tr>
<th>Group</th>
<th>Oramec Day 9</th>
<th>Oramec starved Day 9</th>
<th>Levacide Day 9</th>
<th>Levacide starved Day 9</th>
<th>Levacide starved Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 21</td>
<td>Day 21</td>
<td>Day 21</td>
<td>Day 21</td>
<td></td>
</tr>
<tr>
<td>FHL</td>
<td>91</td>
<td>80</td>
<td>97</td>
<td>85</td>
<td>73</td>
</tr>
<tr>
<td>FUFL</td>
<td>97</td>
<td>90</td>
<td>87</td>
<td>82</td>
<td>78</td>
</tr>
<tr>
<td>MHL</td>
<td>79</td>
<td>36</td>
<td>79</td>
<td>23</td>
<td>26</td>
</tr>
<tr>
<td>MUFL</td>
<td>54</td>
<td>*</td>
<td>76</td>
<td>49</td>
<td>48</td>
</tr>
<tr>
<td>Females</td>
<td>94</td>
<td>86</td>
<td>94</td>
<td>84</td>
<td>76</td>
</tr>
<tr>
<td>Males</td>
<td>66</td>
<td>17</td>
<td>78</td>
<td>39</td>
<td>38</td>
</tr>
<tr>
<td>Combined</td>
<td>83</td>
<td>61</td>
<td>89</td>
<td>74</td>
<td>65</td>
</tr>
</tbody>
</table>

FHL = Females, helminth-line, FUFL = Females, unselected fibre-line
MHL = Males, helminth-line, MUFL = Males, unselected fibre-line
* = FEC post-treatment was higher than that obtained prior to anthelmintic.

6.3.8 Heritability of responsiveness

Heritability estimates calculated using individual mean FEC data were significantly better than zero ($h^2 = 0.37 \pm 0.18$, $p<0.05$). The heritability of a single egg count was much lower but still significant ($h^2 = 0.09 \pm 0.04$, $p<0.05$).

6.4 Discussion

These studies demonstrate that, under the temperate conditions encountered, the resistance of fibre-producing goats to gastrointestinal nematode infection is a heritable phenomenon which, at least in the initial stages of the selection process, has little or no detrimental effect on productivity.

The challenge studies provided encouraging results with selected-line yearlings having lower mean egg counts in both years than the fibre-line animals.
These differences were significant for the females on two occasions in the first year and for the male yearlings over the second season. It was possible to identify HL responder and non-responder individuals with significantly divergent egg counts following artificial and natural challenge. The best male HL yearling responders were kept for use in the breeding programme as described previously (Chapter 4).

The differences between the responses of the male and female HL and UFL groups were not consistent in each season. However this is not surprising. Though a large response to selection was reported in the early years of the *Trichostrongylus* selection programme (Windon *et al.*, 1987), little or no divergence in resistance was recorded over the early years of the *Haemonchus* selection programme (Woolaston, 1990) or the New Zealand *Trichostrongylus* spp. and *Teladorsagia* spp. programmes (Baker *et al.*, 1991).

The responsiveness of sheep to gastrointestinal nematode infection is known to be highly dependent on previous exposure to infection (Gill, 1991; Gray *et al.*, 1990). A number of studies have reported an absence of any development of acquired immunity in goat kids (Le Jambre and Royal, 1976; Kettle *et al.*, 1983; Brunsdon, 1986; Pomroy and Charleston, 1989b; Watson and Hosking, 1989). However, it has been shown that 9-month old Saanen kids develop a high level of acquired immunity to artificial *T. colubriformis* infection (Pomroy and Charleston, 1989a). Pomroy *et al.* (1986) suggest that goats may develop a degree of resistance to nematode infection but that this may not occur until later than 12-months of age. The similarity in FEC levels and the low repeatability estimates calculated in the present study between mean egg count at approximately 3-6 and 12-18 months of age
support the hypothesis that immunity in goats may take some considerable time to develop or may only develop in older animals.

In the current studies there was a marked reduction in faecal consistency in all groups at turnout similar to that seen in the does (Chapter 5). These findings appear to be different to those from previous ovine studies which showed that reduced faecal consistency was associated with host responsiveness (Watson et al., 1986). Studies with grazing sheep in Australia, New Zealand and Scotland suggest that the diarrhoea and fleece dags which accompany nematode infection are largely due to the host inflammatory response and are indicative of the level of larval challenge rather than extant burden (Larsen et al., 1994; Douch et al., 1995a; Suttle and Brebner, 1995). The drop in faecal consistency seen in the current study may be attributable to the intensity of larval challenge and/or changes in diet following the return to pasture rather than host responsiveness since all groups were similarly affected.

The five HL responder yearlings which had been kept for use in the selective breeding programme in the autumn of 1994 were housed in 1995 together with 5 UFL bucks and underwent an identical trickle and single challenge regime to that used in the doe studies (Chapter 5). Mean HL responder FECs after trickle infection were lower than those of the UFL bucks, but much higher than those obtained in previous studies with male and female goats (Chapters 4 and 5, respectively). These differences in egg count following an identical challenge regime illustrate the importance of factors such as seasonally variable factors such as age, previous exposure and nutrition in determining the development and expression of acquired immunity (Gray et al., 1990; Gill, 1991).
Although selecting lines of animals on the basis of an apparent ability to suppress faecal egg output may well produce a desired reduction in pasture contamination, this approach also has some inherent dangers. The principal danger is simply that in selecting for an expression of reduced parasite fecundity one may not necessarily select for other desirable traits such as an ability to regulate worm establishment and development. Individuals lacking these effector mechanisms may be prone to nematodoses since they would theoretically accumulate very large but relatively non-fecund worm populations. The findings from the present study are inconclusive as far as this is concerned. Certainly the FECs during trickle challenge were different between the HL and UFL bucks and the subsequent single challenge study provided no evidence that these differences were attributable to reduced establishment or retardation in the rate of larval development. Post-challenge *T. circumcincta* burdens were 25% greater in HL responders than in UFL bucks and in the latter group slightly more of the established larvae were recovered at the early and mid L₄ stages. The UFL bucks used in the study were selected using ‘normal’ breeding criteria such as appearance and body composition but their responsiveness to gastrointestinal nematodes was unknown prior to the study. This inevitably resulted in considerable heterogeneity in the responsiveness of the UFL bucks which, in studies such as these which use only small numbers of animals, may unduly influence the findings. Further studies are clearly required to investigate which effector mechanisms operate in nematode infrapopulation regulation in selected and unselected goats.

Eosinophil proliferation has been identified as an important component of the host’s inflammatory response towards gastrointestinal nematode infection (Rothwell
and Dineen, 1972; Rothwell, 1989). Though the role of the eosinophil is not fully understood previous studies have suggested that the speed of response may be an important element in host responsiveness (Kimambo et al., 1988; Lawrence and Pritchard, 1994). Though mean peripheral eosinophil levels in the current study were similar, the more responsive individuals tended to exhibit an earlier rise in eosinophil numbers. Circulating eosinophil levels increased significantly faster to significantly higher levels following trickle infection in the selected HL responder than UFL bucks. This was reflected in the former having lower mean egg counts. However, as the selected bucks did not appear to be any better able to regulate larval establishment and development when measured on day 10 after single infection, it may be, as has been suggested (Kimambo et al., 1988) that circulating eosinophils have some accessory function in the mechanisms that suppress parasite fecundity.

There was very little difference in abomasal tissue eosinophil numbers between the breeding HL and UFL males after single challenge. Eosinophil levels were not associated with total *T. circumcincta* burden or the composition of the larval population. Previous sheep studies have offered inconclusive evidence concerning the relationship between tissue eosinophils and the expression of resistance to nematode infection. A positive relationship has been reported from a number of studies (Dineen and Windon, 1980; Douch et al., 1986; Gill, 1991) though positive and zero associations between intestinal eosinophil numbers and *T. colubriformis* burden have also been described (Dineen et al., 1978; Gregg et al., 1978).

Mucosal mast cell and globule leukocyte hyperplasia is a common response of ruminants to nematode infection (Miller, 1984; Rothwell, 1989). Globule
leukocyte proliferation in particular has been closely associated with the
development and expression of acquired resistance in sheep (O’Sullivan and Donald,
1973; Huntley et al., 1984). In the current study there were no differences in MMC
or GL numbers between bucks of the two lines and no evidence of any relationship
with larval establishment or development. This finding differs from those obtained
with the does in the previous study (Chapter 5) which used an identical challenge
regime and where there was an apparent association between MMC and GL numbers
and host resistance. It is impossible to say whether these differences are sex-related
or reflect differences in age and previous exposure. Though a number of studies have
shown a close relationship between resistance and MMC (Rothwell and Dineen,
1972; Alizadeh and Wakelin, 1982a; Gill et al., 1993a) and GL numbers (Huntley et
al., 1984; Stankiewicz et al., 1993), they are not a pre-requisite for immunological
control (Huntley et al., 1992). SMCP concentrations in the two groups on day 10
were not significantly different, nor was there any apparent association with the
expression of resistance. Studies using *H. contortus, T. circumcincta* and *T. colubriformis* have shown the SMCP response to develop rapidly in immune sheep
following challenge (Huntley et al., 1987; Bendixsen et al., 1995). It is likely that
any influence SMCP may have had on the immunoregulatory process occurred prior
to the time of slaughter in this study.

The results of the FECRT illustrated marked differences in drug efficacy
between intact male and female kids and between different anthelmintic families.
Previous goat studies have shown levamisole to be ineffective in controlling a
susceptible *T. colubriformis* population (Gillham and Obendorf, 1985) and to be less
effective than ivermectin against *Ostertagia* spp. and *T. colubriformis* infection
In the current study it was apparent that anthelmintic treatment, particularly levamisole, was considerably less efficacious in males than females. As the male and female kids had been grazed on different pastures prior to housing it is possible that these differences reflected differences in worm populations as well as drug pharmacokinetics. However, the usefulness of the FECRT following different drug treatments may be heavily influenced by variations in the fecundity of those adults which do survive anthelmintic. Scott, Baxter and Armour (1991) showed that the fecundity of surviving multiple resistant \textit{H. contortus} females was higher on day 7 after administration of ivermectin than pre-treatment. In contrast, individual egg numbers were lower following treatment with oxfendazole.

Denying the animals access to feed for the 24 hour period prior to drenching led to a slight improvement in drug efficacy in all groups. The efficacy of many drugs is highly dependant on the length of time that they remain in contact with the parasite. An Australian study (Sangster \textit{et al.}, 1991) demonstrated that extending the delivery period of a dose of oxfendazole increased efficacy in goats against benzimidazole resistant parasites. For drugs such as the benzimidazoles, reducing the rate of digesta flow from the rumen will, by enabling the rumen to serve as a depot, help to increase drug bioavailability and efficacy. Ali and Hennessy (1995a) have shown that halving the feed intake of sheep prior to anthelmintic treatment slowed the digesta flow rate thus extending drug bioavailability. Studies in Australia have demonstrated that halving the feed intake of sheep for 36 hours before and after drug treatment can increase the efficacy of oxfendazole against benzimidazole resistant \textit{H. contortus} and \textit{T. colubriformis} (Ali and Hennessy, 1995b).
The low levels of efficacy calculated from the HL male and female levamisole-treated groups on day 21 suggest that even when administered at 12 mg kg$^{-1}$ this drug may not be particularly effective at removing the established worm populations that predominately consist of *Teladorsagia* spp. The increased egg counts seen on day 21 suggest that drenching with levamisole may have either temporarily influenced female fecundity or have led to the removal of some of the egg-laying females which have subsequently been replaced from the histotrophic larval population. Although the results from this study suggest that there may be some resistance to levamisole, confirmation of resistance unfortunately cannot be made without resorting to an expensive laboratory-based controlled efficacy study. The rapid rate of drug clearance and potential for rumen bypass to occur in goats suggest that this would best be conducted in lambs.

One of the most important remits of any selective breeding programme is that productivity is not adversely affected. As this study was conducted using a commercial cashmere fibre-producing goat herd productivity was measured in terms of body weight and the quantity and quality of cashmere obtained. Previous sheep studies have reported that selection for increased resistance to infection has little or no detrimental effect on bodyweight and wool growth (Albers and Gray, 1986; Albers *et al.*, 1987; Piper and Barger, 1988; Baker *et al.*, 1990; Woolaston, 1990; Bisset *et al.*, 1992). In contrast a recent study in New Zealand recorded a negative relationship between the resistance of Romney sheep to gastrointestinal nematode infection and productivity (McEwan *et al.*, 1992). The results of the present study show that differences in body weight between HL and UFL individuals over the two years were negligible. More importantly, there were no consistent differences in the
quantity or value of fibre produced. As the value of cashmere is a function of fibre colour and diameter, with the highest prices being paid for white, narrow diameter fibres, simply ensuring that selection for resistance does not lead to a lower fibre production is unlikely to be commercially attractive. It is important that factors such as fibre colour and diameter are monitored. Thus, within this study weight of fibre produced and market value were both included as commercially important production parameters. In both years the mean quantity and value of the cashmere obtained from the UFL males was higher than those of the HL though these differences were never significant. In the first season, HL females produced significantly more fibre, with a significantly greater mean value, than did UFL female yearlings. However, in the next year fibre production and mean value were significantly higher in UFL females. There were no differences within the HL yearlings in the quantity or value of cashmere obtained from responders or non-responders.

These kid studies demonstrate that resistance towards gastrointestinal nematode infection in goats is heritable. The heritability estimates calculated for single egg count (0.09 ± 0.04) and individual mean FEC (0.37 ± 0.18) were both significant. The only other study to publish heritability estimates for resistance in goats was conducted using natural *H. contortus* and *T. colubriformis* followed by wholly *T. colubriformis* infection in Fiji (Woolaston et al., 1992b). However, the estimates of heritability obtained were not significantly better than zero (0.04 ± 0.03 in weaners and 0.08 ± 0.06 in adults). The difference in heritability estimates between the studies may be a function of differences in host-parasite interactions between tropical and temperate climates. Though somewhat lower, the heritability
estimates calculated in the present study compare favourably with those quoted for successfully implemented sheep selection studies. Using the mean of five fortnightly FECs, Windon et al. (1987) estimated the heritability of resistance in Merino lambs to infection with *T. colubriformis* to be 0.41 (± 0.19). Heritability of resistance in Merinos to *H. contortus* infection has been estimated using mean FEC data to be 0.33 (± 0.03) (Woolastion, 1990) and using a single measurement at 0.34 (± 0.10) (Albers et al., 1987). The heritability of responsiveness towards *H. contortus* infection in Hungarian Merinos has been estimated to be 0.49 (± 0.17) (Sréter et al., 1994). Studies in New Zealand using naturally infected Romney sheep have provided FEC heritability estimates for a single measurement of 0.33 (± 0.18) (Watson et al., 1986), 0.35 (± 0.12) (Baker et al., 1991), 0.34 (± 0.08) (Bisset et al., 1992) and 0.13 (± 0.07) (McEwan et al., 1992). Heritability estimates calculated using the mean of the FECs before and after anthelmintic were much higher at 0.53 (± 0.15) (Baker et al., 1991). The authors recommend taking the average of two FECs, but suggest that the benefits of taking more samples are outweighed by the costs of conducting the counts (Baker et al., 1991). Stear et al. (1984) have recorded a similar estimate for the heritability of resistance to mixed nematode infection in cattle. These estimates are very similar in magnitude to the heritabilities of production traits such as fleece and body weights for which selection has been very successful.

These studies demonstrate that resistance of Scottish cashmere goats to gastrointestinal nematode infection is heritable and thus is a trait that can be incorporated into a selective breeding programme. The heritability estimates were significant and similar to those reported in previous ovine selection studies and for production parameters. In addition it has been shown that productivity (bodyweight
and cashmere fibre value) was not influenced by selection for increased
responsiveness. At this early date it is unclear for how long selection will have to
continue before there is some significant and permanent divergence between the HL
and UFL lines.
Chapter 7 - General discussion
7.1 General Discussion

These studies have provided useful information on the extent and means by which fibre goats regulate their gastrointestinal nematode populations and also on the potential that exists for selective breeding to enhance the acquisition and expression of resistance. The use of identified responder and non-responder goats has, in a management system where parasite naive animals were not available, provided a valuable model for studying some aspects of infrapopulation regulation and immunoresponsiveness. The challenge model used in these studies has provided information on some of the key density dependant mechanisms involved in caprine nematode infrapopulation regulation. The major density dependant mechanisms regulating gastrointestinal nematode populations in ruminants described by Barger (1987) are those that affect establishment, development, fecundity and persistence of parasitic stages. Barger suggests that these density dependent infrapopulation regulation mechanisms lead to regulation of the suprapopulation and lend stability to ruminant trichostrongylid ecosystems. The anthelmintic disrupted challenge model (ADCM) in which worm burdens were determined 10 days post-challenge obviously only provides data on establishment and development. Some data on fecundity was generated in the male selection studies and the grazing studies have provided useful information on the relative contribution to pasture contamination made by responder and non-responder animals.

The ADCM studies using responder and non-responder does, entire yearling males and the does from the ewe/doe study provided little evidence of a marked capacity in fibre goats to exclude or promptly reject *T. circumcincta* or *T. vitrinus* larvae at an early stage. *T. circumcincta* establishment measured ten days after
challenge ranged from 10.7% to 36.9% with an overall mean recovery of 27.0%.

Indeed if the two groups of does challenged in 1995 in which larval establishment was very low (10.7 and 12.7%) are excluded on the grounds of apparent reduction in the viability of the challenge larvae, then the overall mean rate of establishment increases to 32.1%. Previous sheep studies at Moredun using a similar ADCM and the same strain of *T. circumcincta* (Smith *et al.*, 1983b, 1985, Coop, Huntley and Smith, 1995) have shown that establishment in previously infected lambs and sheep is generally slightly lower, averaging 21.8% with extremes ranging from 7.6% to 39.2%. In the sheep studies it was evident that the expression of resistance against *T. circumcincta* varies with age (Smith *et al.*, 1985) and is influenced by protein availability (Coop *et al.*, 1995). In 10 month old hoggs the mean establishment was 11.1% (Smith *et al.*, 1983b), a figure similar to that recorded in the comparative study (Chapter 3), where the mean establishment in ewes was 7.6%. This study illustrated clearly the differences between the two host species, since the establishment of *T. circumcincta* in identically challenged does of the same age and similar previous exposure to infection was 36.9%. Fibre-goats also had no capacity to limit the establishment or to promptly reject *T. vitrinus* larvae, over 36% of the challenge dose were recovered from does compared to less than 1% in ewes. The apparent inability of goats to regulate incoming larvae was highlighted by the results from the parasite naive lambs used as challenge controls. In adult goats that had had at least two years previous exposure to infection at grazing, more than 36% of the challenge dose became established compared to 26.9% (*T. circumcincta*) and 27.3% (*T. vitrinus*) in young lambs.
Although the ADCM studies show that goats generally have little ability to restrict establishment of *T. circumcincta* comparison between radiolabelled continuous challenge models (CCM, Seaton *et al.*, 1989b) and ADCM (Smith *et al.*, 1983b, 1985; Coop *et al.*, 1995) studies show marked differences in establishment. It is apparent that the use of anthelmintic in ADCM experiments with its obligatory period where the animals have no exposure to challenge, may influence immune exclusion/prompt rejection mechanisms. In the CCM study calculated establishment in lambs exposed to continuous infection for 8 and 12 weeks was 8.9% and 2.0% respectively (Seaton *et al.*, 1989b). These establishment figures are much lower than those recorded in similarly aged lambs used in the ADCM studies, where establishment varied from 19.4-39.2% (Coop *et al.*, 1995) to 22.9-30.7% (Smith *et al.*, 1985). In the most recent study (Coop *et al.*, 1995) the lowest rate of establishment was recorded in lambs given a protein supplement. Since suitable isotopes were not available for the current caprine studies it was not possible to conduct CCM experiments and thus it is possible that exclusion/prompt rejection mechanisms may play some role in natural infections in goats where exposure to infection is more or less constant.

One manifestation of acquired resistance that was evident in the ADCM goat studies was some retardation in the rate of development of established worms. The naive challenge control lambs used in the ewe/doe study provide, assuming of course that no differences exist in the rate of development in goats, some measure of the optimum development rates. In these lambs less than 1% of the *T. circumcincta* challenge was recovered as early and mid fourth stage larvae whereas on average almost 50% of the larval challenge was recovered at these stages of development in
the does and yearling males used in ADCM studies. Moreover no early L₄ were
recovered from challenge control lambs but more than 26% of the larval challenge
recovered from the does and male yearlings used in the ADCM studies were early
fourth stage larvae. Some differences in population structure were also evident in
identified responder animals particularly in the doe studies. In the non-responder
does an average of 30% of the recovered population was at the early L₄ stage and
8.5% at the mid L₄ stage compared to respective figures of 39% and 19% in the
responders. An ability to delay parasite development has obvious consequences for
egg production and hence for suprapopulation regulation. Differences were also
evident in the stage structure of the *T. circumcincta* populations recovered from
entire male responders and non-responders killed 32 days after their last challenge.
The responder animals had a ratio of L₄:fifth/adult stages of 1:6.14 compared to a
ratio of 1:24.85 in non-responders. Whilst the ability to retard development may help
limit egg production, if responsive animals simply accumulate large larval
populations then any subsequent changes within the production system, which are
likely to stimulate the resumption of normal development and maturation may have
serious consequences at both the individual and herd level.

Although there were marked, consistent differences between the egg counts
of grazing responder and non-responder animals that presumably reflect an ability to
regulate their worm populations, it is impossible in these studies, to determine the
relative contribution to egg output made by the various effector mechanisms. Only
the entire adult male responsiveness study provided data on worm fecundity. There
was no evidence of any difference in *per capita* fecundity rates for *T. circumcincta*,
which were 0.062 for responder animals and 0.065 for non-responders. Although
some goat studies (Anon, 1982) suggest that fecundity may be higher in goats than sheep the values for *T. circumcincta* and *T. vitrinus* fall within the range recorded in ovine studies using the same strains (Seaton, 1988; Jackson, 1989). In adult females recovered from lambs killed after 4 and 8 weeks of exposure to either *T. circumcincta* or *T. vitrinus* per capita fecundity tends to decline, in the case of *T. circumcincta* from 0.09 to 0.045 (Seaton, 1988) and for *T. vitrinus* from 0.079 to 0.026 (Jackson, 1989) eggs per gramme of faeces. Responder male goats had a lower fecundity rate (0.025 eggs per female per gramme of faeces) than non-responders (0.076 eggs per female per gramme of faeces). Although the results from the male study show that previously infected adult bucks may have only a limited ability to reduce *T. circumcincta* fecundity it is possible that this mechanism is sex linked and thus may be more apparent in female and wether goats. Entire male goats were evidently more susceptible than females, exhibiting higher egg counts during continuous infection and having higher gastric and intestinal worm burdens than similarly challenged does. Unfortunately none of the doe challenge studies had a design that enabled the recovery of mature female parasites and obviously it was not possible to determine whether there were any effects upon parasite fecundity in does.

Since it was not possible to obtain sufficient responders and non-responders to conduct serial kill studies the current studies have provided no information on death rates of caprine parasites. This is unfortunate, since the more than two-fold differences in *T. circumcincta* and *T. vitrinus* worm burdens recovered from responder and non-responder bucks may well have been attributable, at least in part, to differences in death rate in the two groups of animals.
Most of the findings from the current studies support the view that the mechanisms involved in caprine infrapopulation regulation are poorly developed in comparison to sheep. It would appear that the obvious differences in egg output between responders and non-responders are not attributable to the effects of a single effector mechanism but may have a multifactorial origin.

In the current studies responsive individuals were identified using the simplest of parasitological parameters, faecal egg counts following artificial and/or natural infection. Though FECs may not provide an accurate measure of current worm burden for species such as *T. circumcineta* where egg output follows a markedly stereotypic pattern (Michel, 1969) they were a reliable indicator of the expression of host resistance. However, in the absence of suitable indirect selection parameters, FECs remain an important indicator trait for host resistance as they are relatively easily and cheaply performed and provide a direct measure of the regulation of the parasite suprapopulation (Albers and Gray, 1986).

The use of FEC as the selection parameter for resistance to infection was justified by the significant levels of repeatability of egg count observed in the bucks and does while exposed to natural infection on pasture and following artificial trickle challenge, and the high degree of correlation seen between egg counts following artificial/natural and artificial infection. Caprine resistance to nematodiasis was a comparatively stable characteristic with individuals occupying similar relative positions of responsiveness as determined through FECs on pasture and after trickle challenge and post-challenge worm burdens. In order for resistance to be successfully incorporated into a viable selective breeding programme responsiveness should be expressed towards a range of unrelated parasite species. It is encouraging
that worm burden analysis of the bucks following challenge with *T. circumcineta* and *T. vitrinus* in 1993 and 1994 indicated that responsiveness appeared to be similarly expressed against both species. The results obtained for the bucks and does on pasture and after housing (Chapters 4 and 5, respectively) showed responsiveness to be largely unaffected by season. This was most clearly demonstrated by the responses of the 3 breeding bucks which underwent trickle challenge in 1993 and 1994 as described in Chapter 4. Although there were apparent differences in the results of the two years the animals occupied similar positions of relative responsiveness over the duration of the study. However, the results from the second year of the doe selection programme (1995) suggest that the continued expression of resistance may at times be labile and subject to loss.

Selective breeding programmes have been successfully implemented in Australia and New Zealand to give lines of sheep displaying an enhanced resistance to *Haemonchus, Trichostrongylus* and *Teladorsagia* spp. infection (Woolaston, 1990; Gray, 1991). It is clear from these studies that lambs from the high responder lines are more resistant to nematode infection than are low responder and unselected animals. However these differences did not become apparent for some period of time; up to 5 years in the Australian *Haemonchus* lines (Winton, 1990). The results of the present study suggest that though there was some evidence of differences in susceptibility between the helminth-line and the unselected fibre-line yearlings (Chapter 6) these were not consistent. However as only first generation yearlings were available during the course of the current study large differences were not expected.
The benefits of successfully improving average responsiveness are twofold. Firstly, more responsive individuals shed fewer eggs resulting in a much reduced parasite suprapopulation. Secondly, more resistant animals should require less frequent drenching thus reducing the selection pressure for the development of anthelmintic resistance and helping to extend the life expectancy of the existing drug families. The present studies provided an interesting comparison of the contribution made by responder and non-responder animals to the parasite suprapopulation. Analysis of the FECs of the bucks (Chapter 4) and does (Chapter 5) exposed to natural infection showed non-responders to be shedding approximately twice as many, and responders half as many eggs as the herd mean. Non-responders selected from the helminth-line yearlings had mean egg counts ranging from twice to four times those of selected responders. These results illustrate the potential benefits which might be achieved through selective breeding, either by selectively breeding with high responder individuals or by identifying and removing poorly responsive animals from the breeding herd. The end result of both approaches should be to enhance the average level of resistance of the flock or herd and thus reduce the size of the parasite infra and suprapopulations.

However it is important to consider also the potential drawbacks inherent in selectively breeding for individuals which display high levels of resistance to infection. Ovine studies have reported an increased incidence of fleece dags, as a consequence of diarrhoea, that was associated with enhanced host resistance to nematode infection (Watson et al., 1986). Diarrhoea in naturally infected sheep showing no symptoms of heavy nematode infections has been attributed to the inflammatory response to larval challenge (Larsen et al., 1994; Suttle and Brebner,
Selecting for enhanced responsiveness in fibre goats may in the long term offer some risk of an increasing tendency to scour which in turn may affect both meat and fibre production. Although a seasonal reduction in faecal score was apparent in the current studies there was no evidence that it was associated with responsiveness and was much less pronounced than the scouring commonly seen in lambs and ewes.

There is very little published material describing the inflammatory reactions of goats in response to nematode infection. The results of the current studies highlight interesting differences between the cellular responses of sheep and goats and suggest that although the caprine immune response contains many of the elements seen in ovine inflammatory responses there are quantitative and qualitative differences between the two species. In goats responses to nematodiasis are generally of a much lower magnitude than those seen in sheep.

The results of the doe/ewe comparison conducted at the commencement of this study suggest that though mucosal mast cells may have a similar role within the two species, globule leukocytes play a more active part in the ovine response. Globule leukocyte numbers were considerably higher in does, but appeared to have little influence on worm establishment or development. Indeed the naïve lambs had similar MMC numbers and no GLs, but still harboured fewer worms than the does. Within the male and female goat studies the tendency was for MMC and GL numbers to be inversely associated with nematode burdens and larval development rates. However the absence of any consistent differences in MMC numbers between selected responder and non-responder animals shows that the size of the MMC population is not the most critical element in caprine immunoregulation. The
proportion of total mast cells present as GLs in responder and non-responder male and female goats was surprisingly consistent throughout these studies. This similarity suggests that though GLs may well play a part in the host response they are not responsible for the increased resistance of selected responders. The only conflicting results were those obtained from the preliminary study (Chapter 3) where does had much greater numbers of GLs than ewes. However since the does had significantly greater worm burdens it seems likely that there may be functional differences between caprine and ovine GLs.

Differences in mast cell function between sheep and goats were further illustrated by the relative concentrations of SMCP. SMCP is a pharmacologically active mediator released by mast cells which may have a protective function in nematode infections in sheep (Huntley et al., 1987). SMCP levels detected in goats in all the studies were very much lower than those detected in the challenged ewes (Chapter 3). Previous studies have shown the SMCP response to develop rapidly in immune sheep following *H. contortus, T. circumcincta* or *T. colubriformis* challenge (Huntley et al., 1987; Bendixsen et al., 1995). It is possible that the low SMCP concentrations seen at day 10 post-challenge may reflect differences in the functional activity of ovine and caprine mast cells or that the release of mediators occurs at different times after infection in the two host species.

Blood and tissue eosinophil proliferation is a well documented response of both laboratory animals and ruminants to gastrointestinal nematode infection (Rothwell, 1989). However, the importance of the eosinophil is unclear. Throughout the course of these studies it was noticeable that the peripheral eosinophil response occurred earlier and was more pronounced in selected responder than non-responder
animals of either sex. These findings concur with those of Kimambo et al. (1988) in suggesting that the ability to mount a rapid and vigorous eosinophil response may have an important impact on determining host resistance. The high circulating eosinophil levels detected in responders 3–4 weeks post-challenge support the view that peripheral eosinophilia may be more indicative of the host's immunological responsiveness than the level of nematode infection (Dawkins et al., 1989). Although the association between mucosal eosinophil numbers and resistance to nematode infection is equivocal the increased tissue eosinophil levels detected in selected responder animals in these studies suggest that the eosinophil may be an important component of the local inflammatory response.

There are no published reports of the local humoral and cellular reactions following nematode infection in goats. Those studies which have been conducted with sheep have demonstrated a proliferation in lymphocyte and IgA-containing cells detectable in efferent lymph within 3–4 days of *T. circumcincta* challenge. These cells are thought to be important components of the immunological responses of sheep following nematode infection (Smith et al., 1983b, 1984a). Similar responses, though of a much lower magnitude, were detected in the gastric lymph of goats following single *T. circumcincta* infection. Due to difficulties encountered in maintaining patent cannulations during the immediate post-surgical period, suitable data was only available from a limited number of individuals, but this suggested that local responses occur earlier in the more resistant animals (Chapter 5) but are of a lower magnitude than those seen in sheep.

In order for selective breeding for increased resistance to be economically viable it is important that productivity is not adversely affected. The data collected
during these studies suggest that selection for responsiveness did not have a
detrimental effect on liveweight gain or productivity (Chapter 6). Previous ovine
studies have shown productivity to be unaffected by selection for resistance under
worm-free conditions but that more resistant individuals can maintain higher levels
of productivity when subjected to parasite challenge (Albers et al., 1987). In addition
to reducing the economic losses resulting from nematode infection, more resistant
individuals require fewer drench treatments thus reducing the extra costs of
anthelmintic administration. The drenching regime used at Sourhope which, for most
animals, involved treatments every five weeks during the grazing season (May-
October) was designed for the fibre selection studies. Frequent treatments were used
to ensure that variation in larval challenge between animals grazing on different
areas of hill pasture did not compromise fibre production and hence the genetic
selection programme. The results of grazing studies conducted using the bucks
(Chapter 4) and the second generation kid crop (Chapter 6) where anthelmintic use
was minimal but the responder animals maintained relatively low egg counts suggest
that goats may be more resilient or tolerant of nematode infections than previously
thought. If this is indeed the case then our current caprine control strategies,
including anthelmintic treatment regimes, may need some revision. Further
comparative studies are required in order to improve our understanding of resistance
and resilience in the two host species under experimental challenge with the
prevalent species of nematodes.

Although the results from the current studies support the view that selective
breeding for enhanced responsiveness is possible for fibre goats in temperate
climates, since the acquisition and expression of resistance can never be regarded as
a permanent characteristic then this approach to control is unlikely to provide a
gle solution to the control of nematode diseases. Similar problems may also have
to be faced when nematode vaccines become available since immunity acquired in
this way may also, on occasion, be prone to disruption. One of the dangers inherent
in the development of lines of farm animals which are specifically suited to modern
production systems is that we may overlook, and thus fail to exploit fully, some of
the animal's existing characteristics. In the case of browsing animals such as goats,
for example, it may well be possible to exploit this behavioural characteristic in
order to reduce host parasite contact. The limited capacity of the infective larvae of
most nematodes to migrate vertically suggests that it may be possible either to
develop specific herbage communities or to graze goats only on areas with suitable
species in order to regulate goat infrapopulations.

It is important that research continues to investigate all of the different
aspects of control. The worldwide development of anthelmintic resistance as a
direct consequence of over reliance upon a single method of control provides stark
illustration of the adaptability of helminth parasites. It is evident that future control
strategies will require the integration of appropriate chemical, managemental and
immunological approaches to control if they are to be sustainable.
References


their effects on production and clinical parameters in goats. *Veterinary Parasitology* **40**: 99-112.


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Appendix

*Helminthological Iodine*

250 g Potassium Iodide (Fisons)
50 g resublimed Iodine (BDH chemicals Ltd.)
500 ml tap water

*Tuerk’s White Cell Diluting Fluid*

3% v/v glacial acetic acid
0.01% w/v gentian violet

Diluted in distilled water.

*Carpentier’s Stain*

2% aqueous Eosin Y (Sigma, 0.5 g in 25 ml distilled water)
37% formaldehyde (Sigma) saturated with calcium carbonate (BDH chemicals Ltd.)

Make up saturated formaldehyde by adding 0.5 g CaCO$_3$ per 100 ml formaldehyde. Carpentier’s stain prepared from 2 ml of 2% Eosin Y and 3 ml of 37% formaldehyde added to 95 ml distilled water.

*Phosphate-buffered Saline (PBS) pH 7.4*

200 g NaCl (Fisons)
28.75 g Na$_2$HPO$_4$
5 g KH$_2$PO$_4$
5 g KCl

Made up to 2.5 litres in distilled water. This is a $\times 10$ concentrate of PBS and an appropriate dilution was made before use.

*Carbol chromotrope*

1.0 g Phenol crystals
0.5 g Chromotrope 2R
100 ml distilled water
Melt Phenol crystals in flask by running hot water over the flask. Dissolve Chromotrope in phenol then add water and filter.

**Tissue Emulsifying Buffer**
Titrate 20mM TRIS with HCl until pH is 7.5. Then add 1.5M NaCl and 0.01% azide.

**0.1 M Carbonate/Bicarbonate Coating Buffer pH 9.6**
4.24 g Na₂CO₃
5.04 g NaHCO₃
Make to 1 litre with distilled water and adjust pH as required.

**Citrate-phosphate Buffer pH 5.0 for OPD Substrate**
21.01 g citric acid (0.1 M)
28.4 g Na₂HPO₄ (0.2 M)
Dissolve in 1 litre distilled water. 48.5 ml of 0.1 M citric acid was then added to 51.5 ml 0.2 M Na₂HPO₄ producing a pH approximately 5.0.

**Orthophenylenediamine (OPD) Substrate**
40 μl OPD
40 μl H₂O₂
100 ml citrate-phosphate buffer
Substrate should be prepared no more than 10 minutes before required and H₂O₂ added just prior to use.

**Wash Buffer for Chicken Serum Albumin ELISA**
100 ml ×10 PBS
900 ml distilled water
500 μl TWEEN 20 (Aldrich Chemicals Co. Ltd.)

**Serum/Conjugate Diluent for Chicken Serum Albumin ELISA**
15.79 g Tris-HCl (Sigma)  
29.22 g NaCl (Fisons)  
0.372 g EDTA (Sigma)  
20 g Bovine serum albumin (Sigma)  
30 ml Triton X-100 (BDH Chemicals Ltd.)  
30 ml TWEEN 20 (Aldrich Chemicals Co. Ltd.)  
Make to 1 litre in distilled water

**Tris-buffered Saline (TBS) pH 7.4**  
30.3 g Tris-HCl (Sigma)  
81.5 g NaCl (Fisons)  
30 ml concentrated HCl diluted 1:2  
Make up to 1 litre in distilled water. This produces a \( \times 10 \) concentration of TBS which was diluted with distilled water when required.

**Carbozole Substrate**  
1 ml formdimethyl amide  
3 mg carbozole  
19 ml 0.02 M acetate buffer  
6 drops of 3% \( \text{H}_2\text{O}_2 \)

**Lymph Holding Medium**  
300 ml PBS  
20 ml Pen/Strep  
8000 units heparin

**Leishman’s Stain**  
0.3 g (BDH Chemicals Ltd.)  
200 ml methanol
Evan’s Blue

1 ml Evan’s Blue (Sigma)

99 ml PBS
Publications arising from this thesis


Publications arising from this thesis


A comparison of the mast cell and eosinophil responses of sheep and goats to gastrointestinal nematode infections

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SUMMARY

The mucosal mast cell and eosinophil responses of goats and sheep to a mixed gastrointestinal nematode infection were compared. Groups of eight does and nine ewes, previously maintained on pasture and treated with anthelmintic when they were housed and five worm-free lambs were challenged with 10,000 *Trichostrongylus vitrinus* third stage larvae (*L*$_3$) and 10,000 *Teladorsagia circumcincta* *L*$_3$. Eleven days after challenge, the ewes had significantly (*P*<0.001) lower burdens of abomasal and intestinal worms than the does or naive lambs, but significantly higher (*P*<0.001) tissue concentrations of mast cell proteinase. Toluidine blue-stained sections indicated a paucity of mast cells in the does compared with the ewes, whereas the immunolocalisation of sheep mast cell proteinase revealed similar numbers of stained cells in the two species. This discrepancy was due to the relatively high proportion of globule leucocytes (77 and 91 per cent in the jejunum and abomasum, respectively) in the does compared with the ewes (7 and 24 per cent in the jejunum and abomasum, respectively). No differences were detected between the numbers of circulating or tissue eosinophils in the ewes and does.

GOATS are more susceptible to gastrointestinal nematode infections than sheep (McKenna 1984, Lloyd 1987) and studies carried out in Australia (Le Jambre and Royal 1976, Le Jambre 1984) and New Zealand (Pomroy et al 1986), have shown that when non-lactating ewes and does are grazed together on contaminated pasture, the ewes harbour significantly smaller populations of nematodes. However, it is not clear whether there are underlying differences in the mechanisms by which these two species of ruminant regulate the numbers of gastrointestinal nematodes and there have been few studies of their cellular responses to worm infections. The most striking feature of a gastrointestinal nematode infection is probably the cellular response, which involves an increase in the numbers of eosinophils and mast cells within the mucosa (Miller 1984). The role of these cells remains unclear, but studies in sheep (Huntley et al 1984, 1992) have shown that mucosal mastocytosis, including the presence of intraepithelial globule leucocytes, is invariably associated with gastrointestinal helminthiasis. These and similar findings in rodents (Miller 1971, Alizadeh and Wakelin 1982) have provided evidence that type I immediate hypersensitivity reactions are an important element in the immune-mediated expulsion of worms (Miller 1984).

Earlier studies on the kinetic responses of ovine mast cells were based on the histochemical detection and counting of these cells, while more recent studies have been aided by the purification of a specific mast cell proteinase, termed sheep mast cell proteinase (SMCP) (Huntley et al 1986). Antibodies to this enzyme are localised exclusively in mucosal mast cells (MMC) and globule leucocytes (Huntley et al 1986), and the development of an ELISA for SMCP has made it possible to measure its concentration in gastrointestinal tissues, and this concentration is correlated with the number of MMC and globule leucocytes present in the tissues (Huntley et al 1992). Preliminary observations have revealed that antibodies of SMCP also react with presumptive proteinases in caprine and bovine MMC (J. F. Huntley and H. R. P. Miller, unpublished observations).

The present study took advantage of this inter-species reactivity and compared the mast cell responses of goats and sheep to gastrointestinal nematode infections both immunohistochemically and by analysing the tissue concentrations of mast cell proteinases. The two species were maintained on pasture and subsequently challenged with a mixed infection of *Teladorsagia circumcincta* and *Trichostrongylus vitrinus* larvae. The SMCP analyses were accompanied by a conventional histochemical assessment of the cellular responses in the mucosa, by counting the numbers of MMC and globule leucocytes, and the numbers of local and systemic eosinophils. These cellular responses are considered in relation to the worm burdens carried by the sheep and goats.

MATERIALS AND METHODS

Animals and exposure at grazing

Eight blackface ewes and eight feral cross does aged between two and four years were used; they were rearing twin offspring at the start of the study. The ewes and does were treated with ivermectin (Ivomec, MSD Agvet) at 200 μg kg bodyweight$^{-1}$ before being turned on to pasture in May, and they were drenched with the same dose every five weeks. Rectal faecal samples were collected before the administration of the drug.

Faecal egg counts

The numbers of eggs per gram of faeces were counted by a modified flotation technique, as described by Christie and Jackson (1982), in which all, or one third or one sixth of the eggs recovered from one gram of faeces were counted.

Challenge study

The ewes and does were removed from the pasture in...
RESULTS

Faecal egg counts

The mean faecal egg counts of the ewes and does while they were grazing are shown in Fig 1. Between August and October the does had significantly higher egg counts than the ewes (P<0.001).

Abomasal and intestinal worm burdens

The total numbers of *T. circumcincta* for each animal are shown in Fig 2. The arithmetic mean total worm burden (SEM) was 2690 (451) in the naive lambs, 769 (227) in the ewes and 3687 (649) in the does. The ewes had significantly lower worm burdens than the lambs (P=0.021) and does (P=0.002). Differences were also apparent in the rates at which the worms developed, because in the naive lambs 56 per cent of the challenge dose reached the fifth larval stage, whereas in the does only 25 per cent, and in the ewes only 15 per cent of the dose reached this stage.

The total numbers of *T. vitrinus* in each animal are shown in Fig 3. The arithmetic mean total (SEM) in the naive lambs was 2730 (567) compared with 20 (13) in the ewes and 3631 (448) in the does. The ewes harboured significantly smaller worm populations than either the lambs or does (P<0.001). In the naive lambs 22 per cent of the challenge dose reached the fifth larval stage, compared with 13 per cent in the does and none in the ewes.

Immunodiffusion

Ouchterlony gel diffusion tests with rabbit antiserum to SMCP demonstrated identical single precipitin lines for

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*E.P.G.*

**FIG 1**: Mean (SD) faecal egg counts expressed as eggs per gram (epg) in the ewes and does maintained on pasture between May and September. ***P<0.001

**FIG 2**: Total numbers of *T. circumcincta* recovered from abomasal washings and tissue digests from ewes and lambs, as described in Fig 2

**FIG 3**: Total numbers of *T. vitrinus* recovered from jejunal washings and tissue digests from ewes and lambs, as described in Fig 2

**FIG 4**: Ouchterlony gel demonstrating the precipitin area after diffusion with rabbit antibody to sheep mast cell proteinase (centre well) and supernatants from sheep abomasal (well A) and goat abomasal (well B) or intestinal (well C) homogenates. The precipitin area shows lines of identity between the sheep and goat abomasal tissues indicating antigenic homology between their mucosal mast cell proteinases.
Studies on Caprine Responsiveness to Nematodiasis: Segregation of Male Goats into Responders and Non-responders


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Abstract—Patterson D. M., Jackson F., Huntley J. F., Stevenson L. M., Jones D. G., Jackson E. & Russel A. J. F. 1996. Studies on caprine responsiveness to nematodiasis: segregation of male goats into responders and non-responders. International Journal of Parasitology 26: 187–194. Eighty-three 2–4-year-old intact male goats exposed to a combination of artificial and natural challenge were segregated into responders and non-responders by ranking of weekly faecal egg counts (FECs). Retrospective analysis of samples over a 15-week-period showed responders had a statistically lower mean FEC than non-responders. Estimates of repeatability between consecutive egg counts were significant in both groups. The 6 top responders and bottom non-responders were subsequently given an artificial trickle challenge with Teladorsagia circumcincta and Trichostrongylus vitrinus. Mean faecal egg output was significantly lower in responders than non-responders. Peripheral eosinophil numbers following challenge were significantly greater in responder than non-responder goats. Abomasal and intestinal worm burdens were considerably lower in responders, with evidence of retardation of worm development compared to non-responders. Both abomasal and jejunal tissue eosinophil numbers were significantly higher in responders, although there was no difference in mucosal mast cell or globule leucocyte numbers. These results suggest that under temperate climatic conditions, it is possible to segregate male goats into responders and non-responders on the basis of simple parasitological criteria.

Key words: goats; males; nematodes; responders; non-responders; eosinophils.

INTRODUCTION

Goats are markedly more susceptible to infection with gastrointestinal nematodes than sheep. Comparative studies in Australia and New Zealand found that goats had considerably higher faecal egg output and worm burdens than sheep when grazed together on contaminated pasture, (Le Jambre & Royal, 1976; Pomroy, Lambert & Betteridge, 1986). Moreover, due to species differences in drug pharmacokinetics, anthelmintic treatments may be less efficacious in goats (Sangster et al., 1991) and may, therefore, enhance the rate of selection of drug resistant nematodes (Charles, Pompeu & Miranda, 1989). The high frequency of anthelmintic resistant gastrointestinal nematodes in goats (Varady, Praslicka & Corba, 1994), the potential of goats to seed pasture with large numbers of resistant eggs, and the fact that they share the same nematode species with sheep, mean that goats may pose a significant threat to the economically more important sheep industry worldwide.

The prevalence of anthelmintic resistance in the U.K. and the speed at which it can develop (Jackson, 1993) has been one stimulus for increased research into alternatives to chemotherapeutic control. One encouraging option is to utilize the high degree of
Animal Health and Australian Wool Corporation, Australia.


The Response of Breeding Does to Nematodiasis: Segregation into 'Responders' and 'Non-responders'.


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Running Title: Selection of Responder and Non-responder Does.
INTRODUCTION

The increasing prevalence of anthelmintic resistance among gastrointestinal nematode parasites of small ruminants (Jackson, 1993) is now a major concern for producers throughout the world. The problem is particularly acute in goats which, due to their acknowledged susceptibility to nematode infection (Le Jambre & Royal, 1976; Le Jambre, 1984; Pomroy, Lambert & Betteridge, 1986) are often treated relatively frequently, sometimes using inappropriate dose levels. The differences in drug pharmacokinetics between sheep and goats (Sangster et al., 1991) may be a contributory factor leading to the high nematode prevalence rates seen in goats (Charles, Pompeu & Miranda, 1989). For these reasons, and the fact that they are often grazed together with sheep, goats may play a major role in the spread of anthelmintic resistant nematodes among sheep populations.

Widespread drench resistance, coupled with the time and costs necessary for the introduction of new anthelmintics (Hotson, 1985) and growing public concern over drug residues have in turn led to increased research into sustainable, non-chemotherapeutic control strategies. Currently, a great deal of attention has been directed towards use of the high degree of between-(Stewart, Miller & Douglas, 1937), and within-breed (Albers et al., 1984) variation seen in the immunological responses of sheep to parasite infection. Selective breeding programmes have been successfully implemented in Australia and New Zealand resulting in lines of sheep with increased resistance to *Haemonchus contortus* and *Trichostrongylus colubriformis* infection (Widon, 1990; Woolaston, 1990; Gray, 1991).

Few studies have examined the degree of genetic variation in response of goats to parasite infection, although previous studies in Kenya (Preston & Allonby, 1978) and
France (Richard, Cabaret & Cabourg, 1990) have suggested that genetic variation in resistance to nematodiasis exists. In contrast, a study in Fiji reported very little evidence of genetic variation or repeatability of faecal egg count in goats following natural mixed *T. colubriformis* and *H. contortus* infection and later *T. colubriformis* infection alone (Woolaston et al., 1992). However, a recent study using intact male goats found significant levels of repeatability of FEC after both natural and artificial *Teladorsagia circumcincta* and *Trichostrongylus vitrinus* infection (Patterson et al., 1996).

The aim of the studies described here was to segregate breeding does on the basis of their responsiveness to nematode infection as measured using simple parasitological criteria, namely FEC. These animals would enable comparisons to be made between the cellular responses of responsive and non-responsive individuals to gastrointestinal nematode infection.

**MATERIALS & METHODS**

*Animals and site of study.* These studies were conducted in the Scottish Borders at the Sourhope Research Station of the Macaulay Land Use Research Institute, and at the Moredun Research Institute, Edinburgh. The study herd, containing individuals from widely divergent genetic backgrounds, comprised 95 breeding Scottish Cashmere does, aged between 2-6 years-old and randomly drawn from those animals grazed on the hill pastures at Sourhope. All animals had had previous natural exposure to the nematode species used in these studies.
Faecal egg counts. Faecal egg counts, expressed as eggs per gramme faeces (epg), were carried out using a modification of the floatation technique described by Christie & Jackson (1982).

Identification of 'responders' and 'non-responders'. Animals were segregated into responders and non-responders on the basis of their FECs following naturally acquired infection. The field study was conducted over a period of 12 months, beginning in November 1992, on pasture contaminated predominately with *T. circumcincta* and *T. vitrinus* infective larvae (L₃) (Jackson *et al.*, 1992). Rectal faecal samples were obtained every 5 weeks except for the periparturient period between March and late May. All does were treated with Ivermectin (Oramec MSD Agvet, at 200 μg kg⁻¹ body weight) following sampling. Individual FECs were ranked after each sampling date, the lowest FEC being assigned a ranking of 1 and the highest a value of 95. At the end of the field study a cumulative ranking was obtained. Responder does were deemed to be those with the lowest cumulative FEC ranking, while those with the highest overall ranking were classified as non-responders. The 8 most and 8 least resistant animals, responders and non-responders respectively were selected for further study. All selected animals had live offspring over the period of pasture study.

Infective larvae. Infective *T. circumcincta* and *T. vitrinus* L₃ for artificial infection were cultured from the faeces of sheep carrying pure infections using standard techniques, stored at 4°C and used within 4 weeks of harvesting.

Artificial trickle challenge infection. In November 1993, the selected 8 responder and 8 non-responder does were housed together with 8 does chosen randomly from a control-
The does were housed in one group on straw covered concrete floors under conditions designed to minimise the risk of extraneous infection. All animals were maintained on a diet of ESCA nuts (East of Scotland College of Agriculture, 14% crude protein) and hay and water ad libitum. As the herd has a history of infection with benzimidazole and ivermectin resistant nematodes the does were treated with anthelmintic (Ivermectin at 400 μg kg⁻¹ per day for 10 consecutive days) to ensure removal of all resistant worms. All animals were given an artificial trickle challenge consisting of 2000 *T. circumcincta* and 1000 *T. vitrinus* L₃ orally per day, 5 days per week for 4 weeks commencing 21 days after the last anthelmintic treatment. Rectal faecal samples were obtained from each individual twice weekly up to day 60 after first infection.

**Single challenge infection.** On day 60 all does were treated with anthelmintic (Fenbendazole, Panacur Hoechst Ltd., 5 mg kg⁻¹). Seven days post-treatment (day 67), a single challenge dose of 50,000 *T. circumcincta* L₃ was administered orally to each animal. Ten days post-challenge (day 77) the does were killed and their abomasal worm burdens analysed.

**Eosinophil and total white cell counts.** Venous blood was collected into an EDTA vacutainer tube prior to, and weekly up to day 60 following, trickle challenge infection. Blood eosinophil numbers were determined following fixation in Carpentier's stain as described by Dawkins, Windon & Eagleson (1989). Total blood white cell counts were estimated using a model ZM Coulter Counter (Coulter Electronics Ltd., Luton, UK), calibrated to detect particle sizes down to 3.617 μm diameter. Tissue eosinophil numbers
count and post-challenge analyses were conducted using the untransformed mean value for each animal, and the effect of group tested with the Mann-Whitney test.

RESULTS

Faecal egg counts on pasture

The mean (± SEM) egg counts of the selected 8 responder and 8 non-responder does over the 12 month field study are shown in Fig. 1. Retrospective analysis showed that the mean responder egg count at pasture was significantly lower than that of non-responders (52 ± 5 epg and 234 ± 18 epg, respectively: p<0.001). Mean responder egg count was also significantly less than the herd average of 124 (± 7) epg, p<0.001. Intra-group FEC repeatability estimates conducted on adjacent log transformed FECs were significant for the herd average (0.19 ± 0.05: p<0.01), but not for either the selected responders or non-responders.

Artificial challenge of selected animals

Mean (± SEM) FECs resulting from trickle challenge for each of the 3 groups are shown in Fig. 2. Over this period of artificial infection responders had a mean count of 24 (± 9) epg, significantly lower than that of both the non-responders (252 ± 70 epg, p<0.001) and unselected animals (128 ± 25 epg, p<0.001). Estimates of repeatability conducted on FECs taken twice weekly gave mean values of 0.70 (± 0.05), 0.70 (± 0.06) and 0.74 (± 0.07) for responders, non-responders and unselected animals, respectively. These values are all significant at p<0.001.
Repeatability estimates between mean responder and non-responder FECs on pasture and following trickle challenge were negative (-0.21 and -0.71, respectively). Combining the responder and non-responder counts into one group provided a FEC repeatability estimate between mean natural and artificial infection of 0.67.

**Worm burden analysis**

Mean (± SEM) total *T. circumcincta* numbers are shown in Table 1. The mean responder burden (12,686) was approximately three-quarters that of the non-responders and unselected does (17,744 and 17,613, respectively). This difference was not significant as one of the responders harboured a total burden of 33,000 (a 66% establishment rate) which was considerably higher than for any other animal. Differences in the rates of larval development were also not significant, responders having on average 50% more early and mid L₄, and one-third less late L₄ and fifth-stage worms than non-responder and unselected does.

**Eosinophil counts**

Fig. 3 shows the mean (± SEM) peripheral blood eosinophil counts calculated for each of the 3 groups following commencement of trickle challenge. Eosinophil numbers in responders increased rapidly following challenge, reaching a significantly elevated peak by day 21 post-infection before declining to levels similar to those seen in the other groups. Non-responders showed a two-fold increase in peripheral eosinophil levels up to day 14 post-infection, but this increase was short-lived and not statistically significant. There was no significant change in peripheral eosinophil numbers in the unselected animals over the course of the trickle infection. Mean responder eosinophil counts were significantly higher than those of both the non-responder and unselected groups on days
7 (p<0.01) and 21 (p<0.05) after first infection, and significantly higher than those of the unselected does on day 14 (p<0.05). There were no significant differences in total white cell counts over the period of trickle challenge.

There were no marked differences in peripheral eosinophil numbers during the 10 day period following single challenge infection.

Although non-responders had lower numbers of abomasal tissue eosinophils than responders and unselected does following single challenge, these differences were not statistically significant (see Table 2).

**Mast cell counts**

The numbers of mast cells and globule leukocytes detected in abomasal mucosa are shown in Table 2. Responders had significantly more MMCs than did both non-responder and unselected does (p<0.05). Although responders had twice as many GLs as non-responders and almost four-times as many as the unselected does these differences were not significant. There were no significant differences in either the percentage of total MMCs present as GLs, or the observed concentrations of SMCP between the 3 groups.

**DISCUSSION**

These results suggest that the responsiveness of female goats to gastrointestinal nematode infection, measured using simple parasitological parameters, is a repeatable characteristic largely unaffected by site or mode of infection.

Faecal egg counts have been used in previous studies as the simplest and most obtainable indicator for host resistance to nematode infection, particularly when the
animals are required for future breeding. Although FEC correlates well with total trichostrongyle (Bisset, Vlassoff & West, 1991), and in particular *H. contortus*, burden (Roberts & Swan, 1981), it gives a much less accurate estimate for species such as *Teladorsagia* where egg counts in sheep tend to be markedly stereotypic (Jackson & Christie, 1979). However, the use of FEC as an indicator trait is justified as regulation of egg output is one of the key factors in controlling the parasite supra-population (Albers & Gray, 1986).

The type of challenge used to identify responsive individuals may have an important bearing on the results obtained. Naturally acquired infection has the benefit of taking into consideration aspects of host foraging behaviour and resilience, though results can be confounded by variation in levels of pasture contamination and external stresses such as nutrition and concurrent infection. It is important that animals selected under one regime should be tested under the other to determine if they show similar levels of responsiveness.

Australian studies have tended to make use of artificial infection regimes; superimposed on natural pasture infection in the *H. contortus* programme, and artificial vaccination and homologous challenge in the *T. colubriformis* studies (Windon, 1990). In contrast, studies in New Zealand have employed natural reinfection/anthelmintic disrupted challenge, predominately with *Teladorsagia* and *Trichostrongylus* spp., while on pasture (Baker *et al.*, 1990). It has been suggested that FECs resulting from natural reinfection post-treatment may provide the best indication of host resistance as the counts before and after anthelmintic represent two distinct populations (Baker *et al.*, 1990).

In the current study, retrospective analysis of individual FECs over the 12 month period on pasture enabled segregation of the herd into responder and non-responder
animals. Although repeatability estimates for FEC were not significant in either responders or non-responders, it was encouraging that mean responder and non-responder egg counts were significantly different from each other and the herd mean over the entire period of the grazing study. The low repeatability estimates for responders and non-responders may have been due to a combination of small group size, low individual egg counts, the high degree of variation present in FECs and the anthelmintic-disrupted challenge method used in this study.

An important outcome of this study was that resistance to nematode infection appeared to be a stable characteristic, with animals having consistent rankings of responsiveness during natural pasture infection and when housed and artificially infected. This was reflected in the high repeatability estimates calculated for mean combined responder and non-responder counts on pasture and after housing. Following artificial trickle challenge, mean responder egg count was significantly lower than that of non-responders and unselected animals. Under the controlled conditions of the artificial infection regime FEC repeatability estimates were significantly greater than zero for all groups. Though repeatability estimates may be influenced by both genetic and environmental factors, they indicate the limit of the trait's genetic determination and heritability (Falconer, 1981). The significant estimates obtained in this study suggest that resistance to nematode infection in goats is under genetic control and that it may be possible to breed for increased resistance. These estimates compare favourably with those obtained from previous studies. Barger & Dash (1987) reported FEC repeatability estimates of approximately 0.6 in young Merino wethers which had been housed and trickle infected with H. contortus. Housed studies using female Scottish Blackface lambs given two single infections with 50,000 T. circumcincta separated by anthelmintic treatment, estimated the repeatability in FEC between infections to be 0.3, and the
repeatability for successive samples, taken at 2-3 day intervals, to be 0.75 (Stear et al., 1995). Post-challenge FEC repeatability estimates presented here are very similar to those obtained for entire responder and non-responder bucks which were subjected to an identical challenge regime (Patterson et al., 1996). A previous goat study in the tropics reported very low and insignificant FEC repeatability estimates following natural H. contortus and T. colubriformis infection (Woolaston et al., 1992). The absence of any observed repeatability in the latter study illustrates differences in the host-parasite relationship between tropical and temperate environments.

The analysis of post-challenge worm burdens generally supported the segregation results from the pasture study. The mean responder burden was approximately three-quarters that of non-responders and the unselected group, although this would have been closer to one-half but for the very large count obtained from one of the responder does. This count was larger than any burden found in either of the other groups and illustrates the problems inherent in using faecal egg count as the selection parameter for host resistance. Endoparasitic populations appear to be regulated by an array of discrete or interactively operating effector processes, including those that affect larval establishment, rates of development, persistence and adult fecundity (Barger, 1987). Since this particular individual had consistently low egg counts throughout the study it appears that despite lacking an ability to restrict larval establishment it was able to effectively suppress worm fecundity. Considerable, though non-significant, differences were apparent in the rates of larval development seen in the three groups. In responders, the majority of established larvae were still at the early and mid L4 stages, while many more late L4 and fifth-stage worms were recovered from non-responder and unselected animals. Previous studies in sheep have shown that by day 10 post-challenge the majority of T. circumcincta larvae can be expected to have reached the early adult stage.
(Denham, 1969). As acquired resistance to *T. circumcincta* in lambs is first expressed through a reduction in the rate of development and establishment of infective larvae (Seaton *et al.*, 1989), similar mechanisms also appear to operate in goats.

Although a direct comparison with the responses of selected bucks to a similar trickle challenge regime (Patterson *et al.*, 1996) is not possible due to differences in previous exposure and treatment regimes, these results provide some evidence of differences in susceptibility between male and female goats. In the previous study, mean responder buck egg count between days 21-60 after first infection was comparable to that of the unselected does and considerably higher than that of the selected responder does, while mean non-responder buck FEC was four times higher than that of non-responder does (Patterson *et al.*, 1996). The increased susceptibility if entire males to parasite infection has been reported from a wide range of field and laboratory studies (Barger, 1993).

Identified responder and non-responder individuals provide a useful model for investigation of the immunological responses to infection. The immunoregulatory responses to gastrointestinal nematode infection are highly complex involving a range of specific and non-specific elements (Miller, 1984; Rothwell, 1989).

The association between peripheral and infection-site eosinophilia following helminth infection is complex and is largely age and species dependant (Rothwell, 1989). In this study, mean responder peripheral eosinophilia increased significantly faster than that of non-responders or unselected does after commencement of trickle infection, and was significantly elevated by day 21 post-infection. A number of laboratory studies have suggested that the ability to mount a rapid and vigorous eosinophil response may be an important element in determining host resistance (Handler and Rothwell, 1981; Lawrence and Pritchard, 1994). However, no inter-group differences were detected in
peripheral or abomasal tissue eosinophil levels after single challenge. The results presented here following continuous challenge are similar to those described previously for sheep (Dawkins et al., 1989) and male goats (Patterson et al., 1996), and suggest that high peripheral eosinophil numbers following continuous infection may be a useful indicator of the acquisition of resistance by the host rather than reflecting the level of parasitism.

Mucosal mast cell and globule leukocyte proliferation is a common response of ruminants to gastrointestinal nematode infection (Miller, 1984). This increase in MMC, and in particular GL, numbers has been associated with resistance to T. colubriformis infection in sheep (O'Sullivan & Donald, 1973; Stankiewicz et al., 1993). In the current study, responders had higher abomasal MMC and GL numbers than did either of the other groups, suggesting a possible protective role in the response to nematode infection. Although not directly comparable due to differences in treatment, previous exposure and time of slaughter, it is of interest to note that the abomasal MMC and GL numbers recorded for responder does in this study were considerably higher than those previously reported for randomly selected does (Huntley et al., 1995) and resistant bucks (Patterson et al., 1996). The observation that the proportions of GLs identified in responders and non-responders in this study were very similar, though responders had higher absolute MMC and GL counts and a lower mean worm burden, suggests that the role of MMCs in caprine responsiveness to nematode infection may have both quantitative and qualitative elements. Previous studies with H. contortus, T. circumcincta and T. colubriformis have shown the SMCP response to develop rapidly in immune sheep following challenge (Huntley et al., 1987; Bendixsen, Emery & Jones, 1995). The results of the present study suggest that as all 3 groups had very similar SMCP concentrations,
but very different worm populations, any influence SMCP may have had on the immunoregulatory process occurred prior to the time of slaughter.

The results from this and a previous study using entire male goats (Patterson et al., 1996) demonstrate the feasibility of using simple parasitological criteria to select responsive Scottish Cashmere goats that may be used in a selection programme to enhance responsiveness to gastrointestinal nematodes. The high levels of anthelmintic resistance in non-dairy goats in the UK (Jackson et al., 1992) suggest that this approach for the control of nematodoses may prove to be a key element in the development of integrated and sustainable control regimes for goats which are not wholly reliant on intensive chemoprophylaxis.

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Table 1: Mean (± SEM) *T. circumcincta* worm burden at day 10 post-challenge for responder, non-responder and unselected does including mean percentage of established worms recovered at 5th stage of development.

<table>
<thead>
<tr>
<th></th>
<th>Total Burden</th>
<th>% at 5th Stage</th>
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<tbody>
<tr>
<td><strong>Responders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>12,686</td>
<td>1</td>
</tr>
<tr>
<td>SEM</td>
<td>(3946)</td>
<td>(0)</td>
</tr>
<tr>
<td><strong>Non-responders</strong></td>
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<td></td>
</tr>
<tr>
<td>Mean</td>
<td>17,744</td>
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</tr>
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<td>SEM</td>
<td>(1556)</td>
<td>(4)</td>
</tr>
<tr>
<td><strong>Unselected</strong></td>
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<td></td>
</tr>
<tr>
<td>Mean</td>
<td>17,613</td>
<td>7</td>
</tr>
<tr>
<td>SEM</td>
<td>(2473)</td>
<td>(3)</td>
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</table>
Table 2: Mean (± SEM) tissue eosinophil (Eos), mucosal mast cell (MMC), globule leukocyte (GL) and total mast cell numbers per mm² abomasal tissue, and sheep mast cell proteinase (SMCP) concentrations (µg g⁻¹ wet tissue) for responder, non-responder and unselected does.

<table>
<thead>
<tr>
<th></th>
<th>Eos (± SEM)</th>
<th>MMC (± SEM)</th>
<th>GL (± SEM)</th>
<th>Total (± SEM)</th>
<th>% GLs (± SEM)</th>
<th>SMCP (µg g⁻¹) (± SEM)</th>
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</thead>
<tbody>
<tr>
<td>Responders</td>
<td>13.8 (± 3.2)</td>
<td>75 (± 11)</td>
<td>30 (± 8)</td>
<td>105 (± 17)</td>
<td>28.0 (± 6.5)</td>
<td>1.60 (± 0.34)</td>
</tr>
<tr>
<td>Non-responders</td>
<td>6.4 (± 2.4)</td>
<td>43 (± 8)</td>
<td>15 (± 5)</td>
<td>58 (± 9)</td>
<td>23.7 (± 6.8)</td>
<td>1.16 (± 0.06)</td>
</tr>
<tr>
<td>Unselected</td>
<td>15.8 (± 3.5)</td>
<td>41 (± 7)</td>
<td>5 (± 3)</td>
<td>46 (± 7)</td>
<td>12.1 (± 7.1)</td>
<td>1.76 (± 0.39)</td>
</tr>
</tbody>
</table>

Figures with different superscripts are significantly different at p<0.05.
Fig. 1: Mean (± SEM) FECs for selected 8 responder and 8 non-responder does following natural pasture infection.

Fig. 2: Mean (± SEM) responder, non-responder and unselected does FECs following commencement of trickle challenge.

Fig. 3: Mean (± SEM) peripheral eosinophil numbers for responder, non-responder and unselected does over the period of trickle challenge.
1. To 20 Days Post-challenge

Graph showing E.P.G. ( Eggs Per Gram ) over Days Post-challenge. The graph includes lines for:
- Responders
- Non-responders
- Unselected
Eosinophils $2.5 \times 10^9 \text{ l}^{-1}$

Days Post-challenge