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Determinants of resistance to Nematode infection in Scottish Blackface sheep

By

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A thesis submitted for the degree of doctor of philosophy in the Faculty of Veterinary Medicine, University of Glasgow

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DECLARATION

I declare that the work presented in this thesis has been carried out by me, and has not been submitted to any other degree.

The QTL studies in Chapter six were carried out in conjunction with Miss Gail Davies, Roslin institute, Edinburgh. Any other collaboration and assistance has been duly acknowledged.

Mohamed A. Benothman

Some of the work described in this thesis has been the subject of the following publication:

Variation among faecal egg counts following natural nematode infection in Scottish Blackface lambs. Stear, M.J.; Abuargob, O; Benothman, M; Bishop, S.C.; Innocent, G.T.; Kerr, A.; Mitchell, S. Parasitology

Quantitative trait loci associated with parasitic infection in Scottish Blackface sheep. Davies, G.; Stear, M.J.; Benothman, M.; Abuargob, O.; Kerr, A.; Mitchell, S.; Bishop, S.C. Heredity (in Press)

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TABLE OF CONTENTS

List of Figures

List of Tables

Abbreviations

αβ T cell	alpha beta T cell
Ca	calcium
Cl	chloride
°C	degrees Celsius
cm	centimetre
cM	centimorgan (A unit of crossover frequency in linkage maps of chromosomes equal to one hundredth of a morgan).
CO_2	carbon dioxide
CP	crude protein
Cu	copper
D. flagrans	Duddingtonia flagrans
Da	Daltons
dH_20	distilled water
E. coli	Escherichia coli
EDTA	disodium ethylene diamine tetracetic acid
EGTA	ethylene glycol tetracetic acid
ELISA	enzyme linked immunosorbent assay
FCS	foetal calf serum
Fe	iron
FEC	faecal egg count
γd T cell	gamma delta T cell
g	grams
GL	globule leucocyte
GM-CSF	granulocyte macrophage colony stimulating factor
H. contortus	Haemonchus contortus
hr	hours
IFN-γ	interferon gamma
IgA, D, G, E, M	immunoglobulin, subclasses A, D, E, G and M
IL-1, -2 etc	interleukin-1, -2 etc
K	potassium
kDa	kilo Dalton
kg	kilograms
L. donovani	*Leishmania donovani*
L. monocytogenes	*Listeria monocytogenes*
L. major	*Leishmania major*
L_1	first stage larvae
L_2	second stage larvae
L_3	third stage larvae
L_4	fourth stage larvae
λ	lambda
ln	natural logarithm
log10	logarithm to the base 10
LP	low protein
M	molar

Mg	magnesium
mg	milligrams
MHC	major histocompatibility complex
min	minutes
µg	micrograms
µM	micromolar
µm	micrometre
ml	millilitres
mM	millimolar
MMC	mucosal mast cell
mm	millimetre
mRNA	messenger RNA
N. americanus	*Necatur americanus*
N. battus	*Nematodirus battus*
N. brasiliensis	*Nippostrongylus brasiliensis*
N. filicollis	*Nematodirus filicollis*
N. spathiger	*Nematodirus spathiger*
Na	sodium
NEM	N-ethylmaleimide
no.	number
T. circumcincta	*Teladorsagia circumcincta*
O. ostertagi	*Ostertagia ostertagi*
OD	optical density
OLA	ovine lymphocyte antigen
%	percent
P	phosphorous
P	probability
P. falciparum	*Plasmodium falciparum*
PBS	phosphate buffered saline
PBS-T	PBS + Tween 20
PBS-TSM	PBS + Tween 20 + skimmed milk powder
PMSF	phenyl methyl sulphonyl ketone
r	correlation coefficient
rpm	revolutions per minute
RT-PCR	reverse transcriptase-polymerase chain reaction
S. japonicum	*Schistosoma japonicum*
S. mansoni	*Schistosoma mansoni*
S. stercoralis	*Strongyloides stercoralis*
SDS	sodium dodecylsulphate
S.E.	standard error of the mean
spp.	species
T. axei	*Trichostrongylus axei*
T. circumcincta	*Teladorsagia circumcincta*
T. colubriformis	*Trichostrongylus colubriformis*
T. gondii	*Toxoplasma gondii*
T. trifurcata	*Teladorsagia trifurcata*
T. vitrinus	*Trichostrongylus vitrinus*
TPCK	N-tosylamide-L phenylalanine chloromethyl ketone
UK	United Kingdom
W. bancrofti	*Wuchereria bancrofti*

Abstract

Gastrointestinal parasitism is one of the greatest causes of disease and lost productivity in domestic animals .It remains a major constraint on production and welfare in domestic animals in all geographical locations all over the world. Anthelmintic treatment is the mainstay of current control methods. However, with the widespread use of anthelmintics, the problem of parasite resistance has emerged. Other modalities of prevention and treatment are urgently needed. One of the most promising developments in this respect is the use of genetically resistant sheep. Selective breeding of animals resistant to gastrointestinal parasitism is particularly attractive, but identifying the phenotypic and genetic markers of resistance on which selection will be based is a major problem. Hence, this thesis has investigated some of the phenotypic and genetic determinants of resistance to nematode infection in Scottish Blackface sheep when naturally infected with the gastrointestinal nematodes, particularly *Teladorsagia circumcincta*, and how these determinants may facilitate the successful selection of resistant animals.

Chapter three has investigated the seasonal pattern of *T. circumcincta* infection, as well as the changes in the mean and the distribution of faecal egg counts in Scottish Blackface sheep. There was no discernible pattern to egg counts with each season, and this could be attributed to factors like changes in grazing management, anthelmintic treatment, weather and humidity.

Work described in chapter four has shown that there was considerable variation among populations. Faecal egg counts vary in naturally infected sheep, and mean egg counts vary among different populations and among the same population sampled at different times. In addition high mean faecal egg counts are not necessarily due to high intensities of infection, but probably reflect the contribution of species other than *T. circumcincta*.

The present study has not found any correlations between plasma IgA activity against third-stage larvae of *T. circumcincta* and faecal egg (chapter five). Moreover, there was no significant correlation between growth rates in 24 week old lambs. The results suggest that IgA activity against fourth-stage larvae is probably a better marker of nematode resistance than IgA activity against third-stage larvae.

This research has identified three new sequences at MHC class II *DRB1* gene of Scottish Blackface sheep (chapter six). In addition, the study has provided evidence for QTL linked to parasitic infection and immune response on chromosome 3 and chromosome 20. The results reached in this study suggest that some aspects of parasite resistance are under strong genetic control, and with further research, this information could be used to select sheep for increased resistance to parasitic infection in marker assisted selection scheme.

The work detailed in this thesis has further increased our understanding of the complex host/parasite relationship, and has confirmed that selective breeding, using the various phenotypic and genetic markers studied, is possible.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Gastrointestinal Parasitism on sheep

1.1.1 General

Sheep production constitutes an important part of livestock farming in many parts of the world today. For example, in the UK there are approximately 20 million ewes and 24 million lambs (Clarkson and Winter 1997). The estimation of the sheep population in the world in 2002 is slightly over 1000 million. This number has not changed greatly from year to year, but there are significant changes between developed and developing countries (FAO-STAT 2003).

World wide, parasite infections are one of the greatest causes of disease and lost productivity in domestic animals (Vercruysse and Claerebout 2001).they can affect the host animal in many ways, for example, damage to the gut causing poor digestion or uptake of nutrients by the parasite, resulting in the host suffering from nutritional deficiencies. In general, the damage inflicted upon the host by the parasite is caused by a combination of events, the animal therefore exhibits decreased production, which is usually observed as a decreased growth rate and decreased wool weight, and if left untreated would cause unacceptable welfare problems (Coles 1998). Sales of antiparasitic drugs have grown more than in any other sector of the world wide animal health market in the last decade and now represent one quarter of the $18 billion market (Dalton and Mulcahy 2001). Parasites can infect and affect most internal and external niches of the body but nematode infections of the gastrointestinal tract of ruminants are particularly important. Table 1 lists the common nematode infections in sheep. This introduction will focus on gastrointestinal nematode infection in general and on *T. circumcincta* in particular in Scottish Blackface sheep.

Table 1.1 Nematode parasites in sheep

Parasite	Site	Regional Distribution
Haemonchus contortus	Abomasum	Tropical/sub-tropical
Teladorsagia circumcincta	Abomasum	Temperate/sub-tropical
Ostertagia trifurcata	Abomasum	Temperate/sub-tropical
Ostertagia leptospicularis	Abomasum	Temperate/sub-tropical
Trichostrongylus axei	Abomasum	Worldwide
Trichostrongylus colubriformis	Small intestine	Worldwide
Trichostrongylus vitrinus	Small intestine	Worldwide
Cooperia curticei	Small intestine	Worldwide
Cooperia surnabada	Small intestine	Worldwide
Nematodirus battus	Small intestine	Temperate
Nematodirus filicollis	Small intestine	Temperate
Nematodirus spathiger	Small intestine	Temperate
Bunostomum trigonocephalum	Small intestine	Worldwide
Gaigeria pachyscelis	Small intestine	Tropical
Strongyloides papillosis	Small intestine	Worldwide
Trichuris ovis	Large intestine	Worldwide
Chabertia ovina	Large intestine	Worldwide
Oesophagostum columbianum	Large intestine	Tropical/sub-tropical
Oesophagostum asperum	Large intestine	Tropical/sub-tropical
Oesophagostum verulosum	Large intestine	Tropical/sub-tropical

1.1.2 *Teladorsagia circumcincta*

1.1.2.1 Description

T. circumcincta was previously classified in the superfamilly Trichostrongyloidea of the order Strongylida, and the class Secernetea of the Nematoda phylum. However, in a more recent classification based on small subunit ribosomal RNA (SSU rRNA) phylogenetics, nematodes classified into five major clades: Dorylaimia (clade I), Enoplia (clade II) and Chromadorea (which includes Rhabditida). Rhabditida can be further divided into Spirurina (clade III), Tylenchina (clade IV) and Rhabditina (clade V) (Fig .11) , *T. circumcincta* is classified in the order Strongylida in clade V of the phylum Nematoda (Blaxter et al. 2000;Parkinson et al. 2004). *T. circumcincta* parasitises the abomasum of small ruminants and disease is associated with the emergence of the late L_4 from the gastric gland. Adults appear light brown and measure 0.6-1.2 cm. Males have long slender spicules with three distal branches (Soulsby 1986).

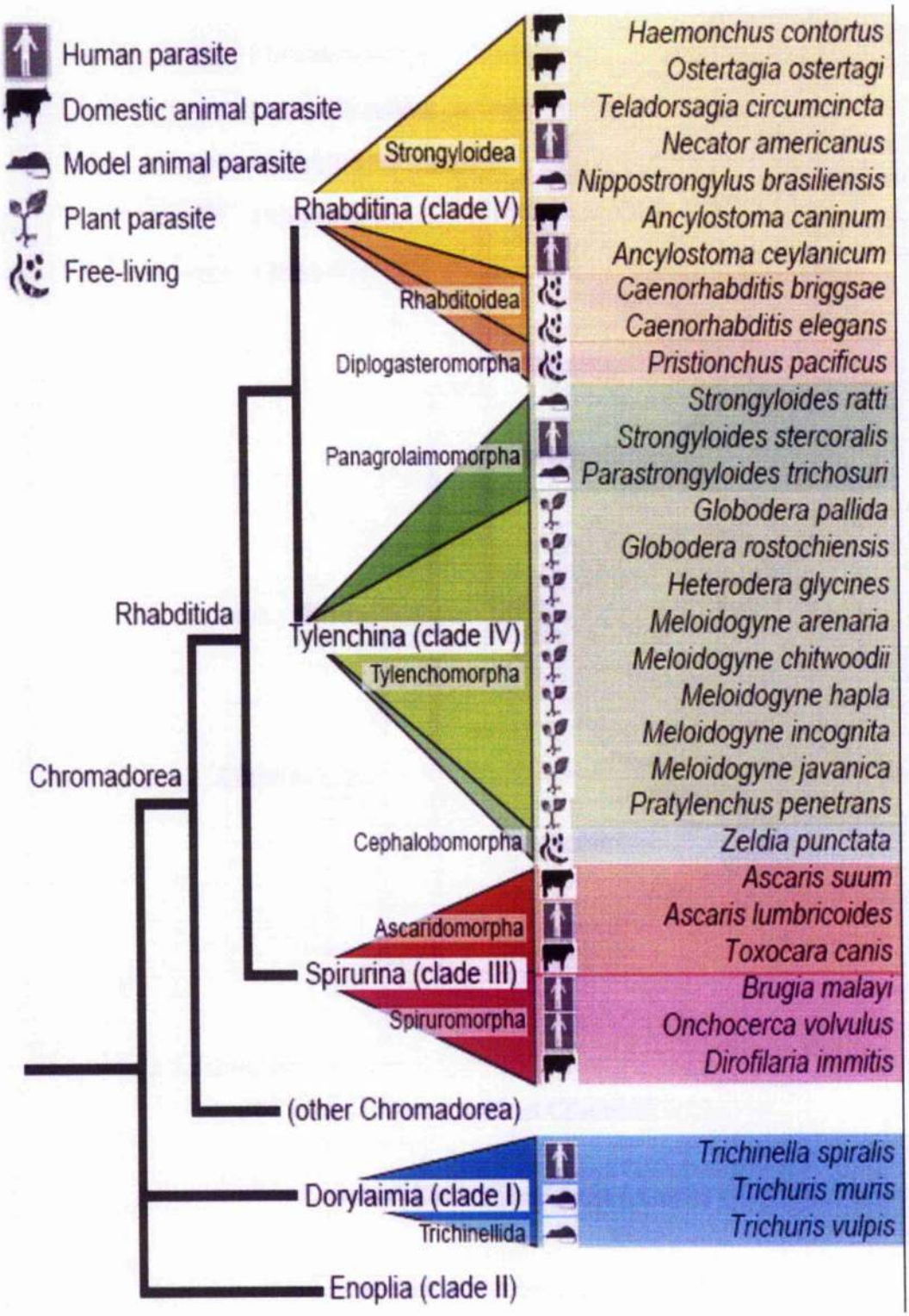


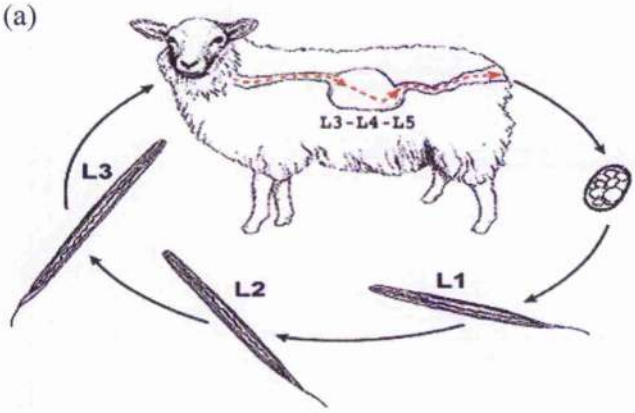
Figure 1.1. The phylogenetic structure of the Nematoda revealed by analysis of full-length small subunit rDNA sequences.

1.1.2.2 Life cycle

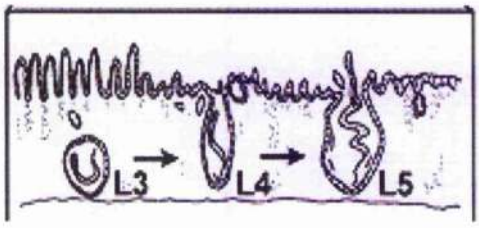
T. circumcincta has a direct life cycle (no intermediate host) as shown in Figure 1.2 and involves development through a series of cuticle shedding moults. There are three post-hatching pre-parasitic stages L_1, L_2 and L_3 and two parasitic stages L_4 and a final L_5 immature adult stage (see Figure 1.2a). Hatched larvae become infective (L_3 larvae) in about 6 to 7 days and most larvae survive on pasture for 4 months, although some larvae may survive much longer. Infection is by ingestion of L_3 larvae by the host while grazing. The minimal prepatent period is about 14 days (Stear et al. 1995d), but can be up to 3 months (Stear et al. 1995a).. The L_3 larvae penetrate the lumen of the abomasal gland and moult to the L_4 stage, as shown in Figure 1.2b and 1.2c. Following a period of growth and a further moult to the L_5 (immature adult) stage, they emerge from the gland and mature on the mucosal surface. Male and female worms copulate producing eggs, which are passed by the host in the faeces. The eggs hatch to the L_1 stage and further development to the L_2 and L_3 stages on pasture. Typically this life cycle takes three weeks to complete with variation depending on the weather and immune status of the animal (Urquhart et al. 1996).

Figure 1.2. The lifecycle of a typical stomach worm. L1-L3 larval stages are free-living on pasture. L3 are ingested while the sheep is grazing and migrate to the abomasum and burrow into the abomasal wall. L4 and L5 stage development occurs within the abomasal gland from where the larvae emerge and copulate producing eggs. (b) In the mucosa the L3 develops into the L4 and L5 and then emerges. (c) The developing L4 larval stage within the mucosal layer of the abomasum (from: http://cal.nbc.upenn.edu/merial/ Nematodes/nems_9.htm).

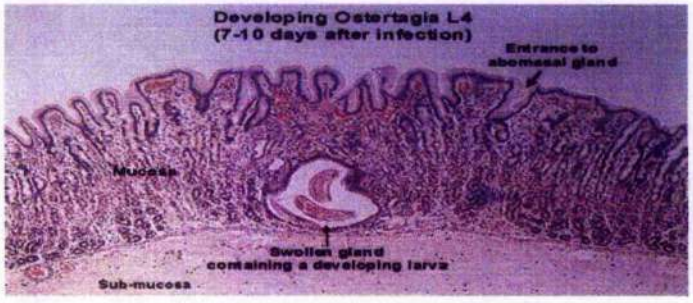
(a)



(b)



(c)



1.1.2.3 Epidemiology

In the temperate regions, eggs are passed in the faeces of lambs during the spring, reaching a peak in the late summer (Boag and Thomas 1977). The source of the infection for lambs is believed to come from overwintered larvae, eggs passed by ewes during the pre-parturient period, and from lambs developing patent infection (Boag and Thomas 1977). Thus, pasture contamination builds up during the summer to give clinical teladorsagiasis. After October the majority of ingested larvae become arrested. These arrested larvae can subsequently develop into adults during the following spring.

1.1.2.4 Pathogenesis and clinical signs

Disease is thought to be a consequence of the presence of fourth-stage larvae within the gastric gland (Armour et al. 1966). Infected gastric glands become stretched as the larvae grow and the surrounding epithelium becomes hyperplastic. Partial cells are replaced by undifferentiated epithelial cells, leading to a reduction of functional gastric gland mass. This de-differentiation occurs not only in infected glands but also in adjacent uninfected ones. The loss of parietal cells leads to an increase in abomasal pH and a failure to convert pepsinogen to pepsin and thus, to a reduction in digestive efficiency.

There is also evidence that infection stimulates zymogen cells to secrete pepsinogen. Elevated levels of plasma pepsinogen can be detected by infected animals. This may be due either to leakage across the damaged mucosa or direct secretion into the circulation (McKellar 1993). The elevation in pH can also lead to a failure of bacteriostasis.

The mucosa of affected animals is oedemic and hyperaemic with occasional mucosal sloughing. At the cellular level, there is a leakage of plasma protein across the mucosal membrane, which may be due to a breakdown or incomplete formation of intracellular

junctions. This is the main cause of the reduced nitrogen digestibility seen in infected animals (McKellar 1993).

Teladorsagiasis in lambs rarely causes diarrhoea. The main clinical signs are a depression in feed intake and loss of weight. Infection leads to reduced nitrogen digestibility, reduced calcium and phosphorus deposition, poor carcase conformation, and impaired wool growth (Sykes and Coop 1977). Thus, the economic impact is through a depression in productivity. High levels of infection have been associated with population crashes in feral sheep on St. Kilda (Gulland 1992). Those animals that survived population crashes were less heavily parasitised than those that died. Whether these deaths can be directly attributed to parasitism is uncertain, but even a moderate experimental infection can reduce growth rate of the host by approximately one third (Coop et al. 1985).

1.2 Control of gastrointestinal nematodes of sheep

1.2.1 Anthelmintics

Anthelmintics have a strong effect in limiting worm burdens, and in particular in controlling adult worms. However, eggs can be found in faeces within 14-28 days after drug treatment coming from fresh infections or from worms that survive treatment (Bishop et al. 1996).

The development of resistance to anthelmintics is a major threat to parasite control worldwide (Waller 1994). Initially, resistance developed slowly against the less efficient early benzimidazoles, but with the introduction of more efficient anthelmintics, selection pressure for resistance has increased. At present, there is resistance to pharmaceuticals within each of the anthelmintic groups available for treatment of some parasite species. There has been no convincing evidence that if selection pressure is removed there is a reversion to susceptibility

to anthelmintics (Jackson 1993). This is supported by findings that parasites resistant to benzimidazoles are as fit as susceptible parasites from the same strain (Elard et al. 1998).

For strategies aimed at delaying the onset of resistance to be successful they must use a minimum of chemoprophylaxis in order to reduce the number of parasite generations exposed to anthelmintic, while maximising the efficacy of the drug in order to remove heterozygous resistant genotypes. An important concept when considering this is the relationship between the parasite population within an animal (the infrapopulation) and the nematode population on the pasture (suprapopulation). If there is a large infrapopulation and a small suprapopulation and the host is wormed frequently then there will be a rapid increase in the number of resistance alleles within the total worm population. For instance, a dose and move strategy where animals coming from a highly contaminated pasture are treated and then moved onto a clean pasture is likely to select for parasite resistance (Sutherland et al. 2002).

In the UK control of parasitic gastroenteritis is achieved by strategic dosing with anthelmintics (Mitchell and Fitzsimons 1983) or clean grazing, or a combination of both. Strategic dosing involves treatment of the ewes during the peri-parturient period, to limit pasture contamination, followed by dosing of the lambs during the grazing period. The frequency and timing of the strategic dosing of lambs depends on the risks of nematodirosis and the stocking rate of the pasture. One of the most important sources of infection for lambs is the peri-parturient rise in faecal egg output in ewes during the last trimester of pregnancy and early lactation (Armour and Coop 1991). This appears to be due to a relaxation in the immune response to parasites resident within the abomasum and to freshly acquired larvae.

Other strategies aimed at increasing the parasite kill are the use of split doses of anthelmintic or combination therapy. Splitting the dose over two days increases the efficacy of

benzimidazoles drugs as it is related to the length of time the parasite is exposed to the drugs (Sangster et al. 1991). Combination therapies are based on the premise that it is highly unlikely that resistance alleles to two groups of anthelmintics will be found on one parasite. Such a strategy has been shown to delay the emergence of resistance compared to strategies employing single drug therapies (Jackson 1993). However, simultaneous resistance to both classes may emerge at the same time with both strategies. A strategy employing two or more anthelmintics would add to the cost of production and may not be economically feasible. At best, these strategies will only delay the emergence of resistance.

Perhaps one of the largest contributors to resistance emergence is the frequent underdosing of animals. Underdosing selects for heterozygous resistance thus increasing the number of resistance alleles within a population. The dose given should be that dose needed for the heaviest lamb but often a lower average dose is given thus underdosing the heaviest lambs (Boag and Thomas 1973).

1.2.2 Grazing management

Good grazing management is making the most efficient use of herbage grown on a farm. Grazing management techniques can offer relatively simple and rapid solutions for improving helminth control and reducing anthelmintic usage (Barger 1997). Clean grazing is the practice of grazing sheep and lambs on pasture that has not carried young sheep or pre-parturient ewes during the previous 12 months. On many sheep farms clean grazing is restricted and often reserved for the lambs after weaning and dosing.

Alternate grazing of pastures by sheep and cattle is a way of preparing clean pastures; for young sheep, by pre-grazing with cattle or for young cattle, by pre-grazing with sheep (Barger 1996). Most Nematode larvae of sheep die out during spring (Boag and Thomas 1970).

Therefore, it is possible to exchange pastures grazed by sheep with those grazed by cattle. Moving newly weaned lambs on to clean pasture, i.e. pasture not grazed by sheep in the previous season, before the expected mid-summer rise in herbage infection has also been shown to be effective in reducing parasite loads and improving production (Githigia et al. 2001). The same authors also reported that, contrary to some other reports, the benefits of moving lambs to clean pasture could be achieved whether the move was accompanied by anthelmintic treatment or not.

Mixed grazing of cattle and pigs also favours the reduction of *O. ostertagi* larval levels on pasture (Fernandez et al. 2001). The reduction in larval numbers is mainly due to the grazing behaviour of the pigs, which, by grazing up to the very edge of the cattle faeces, will either expose the larvae in faeces to adverse environmental summer conditions or ingest the cattle larvae, or both. Another study replaced a community of sheep endoparasites that had been classified as resistant to levamisole and albendazole with a community of more susceptible parasites using a dilution approach (Bird et al. 2001). Strategically timed anthelmintic treatments combined with pasture management reduced to non-detectable levels the endemic community of anthelmintic resistant parasites in the flock.

Crops can be alternated with grassland and if cattle are available there could be a rotation of cattle, sheep, and crops (Armour and Coop 1991). Strategies dependent on management alone are seldom practical as 'clean' pasture is rarely available. However, theses strategies are only applicable *to T.* circumcincta which infect sheep only. In addition, theses strategies can occasionally lead to parasitic gastroenteritis from those parasites such as *T. colubriformis, T. axei* and *N. battus* that are capable of crossing the species barrier between cattle and sheep. Also, there is evidence that a small proportion of infective larvae can survive beyond 2 years on the herbage and upper soil layers (Armour et al. 1980). Therefore, there is a risk that

reliance on pasture management alone may fail. Pasture that has been free from sheep for 12 months can still harbour sufficient larvae to allow for sufficient numbers to develop during the subsequent grazing year to allow the appearance of clinical teladorsagiasis (Boag and Thomas 1970).

The major problems of this method of parasite control are the levels of planning required and the fact that pastures cannot be used for grazing for extended periods, thereby limiting the numbers of animals a rotational grazing scheme could sustain. This obviously introduces economics into the equation. However, this method of control may be more cost effective than treating higher numbers of animals with anthelmintics. Grazing management is definitely a viable alternative to anthelmintic treatment, although further research is required for other control strategies.

1.2.3 Vaccination

Vaccines have proven to be an extremely useful means of protection against a wide range of microorganisms. Vaccination against helminth parasites of animals has recently been reviewed, and much of the major work on the development of vaccines to gastrointestinal nematodes has been discussed (Emery 1996;Newton and Munn 1999).

Vaccines are safe, leave no chemical residues (and therefore there are no with-holding periods for animals), are environmentally friendly and will be acceptable to consumers and users alike who are already familiar with the concept of vaccination in human medicine (Dalton and Mulcahy 2001). While there are some vaccines available against toxoplasmosis in sheep and the tick *Boophilus microplus*, there are no commercially available vaccines for the control of helminth infections in ruminants, with the exception of that for the bovine lungworm, *Dictyocaulus viviparus* (Smith 1999).

It is unlikely that antiparasite vaccines will attain the almost 100% efficacy associated with new anthelmintics and bacterial/viral vaccines, but evidence obtained by using computer models of the population dynamics of host-parasite interactions indicate that adequate control can be achieved with vaccine efficacies well below 100% (Knox 2000). Modelling has demonstrated that a vaccine giving 50% protection at weaning to all sheep was about as effective as a strategic drenching program at controlling worms (Barger 1996). With a conventional vaccine, excellent control of *T. colubriformis* was achieved with 60% efficacy in 80% of the flock. And with a novel antigen vaccine, results were better than strategic drenching programs if there was better than 80% efficacy or 80% of the flock responded with 60% protection or better (Barnes et al. 1995).

Attempts have been made to immunise animals with vaccines made from a variety of parasite antigen preparations. Whole and gamma irradiated larval preparations have successfully been used to immunise mature (> 6 months), but not young animals (Knox 2000). Recent work has concentrated on isolating and identifying specific immunogenic parasite proteins, both somatic and excretory/secretory, which might act as suitable vaccine agents. Antigens that are preferentially recognised by resistant animals could also provide additional markers for the breeding of more resistant lines of animals and this is described later.

Mcgillivery et al. (1992) extracted and isolated a 31 kDa glycoprotein antigen from the infective L_3 stage of *T. circumcincta* that was recognised by total antibody in sera of infected sheep as early as 3 weeks after experimental infection. The same group then reported that the purified 31 kDa antigen had been used to successfully immunise lambs against challenge infections with *T. circumcincta* (Mcgillivery et al. 1992) but this could not be confirmed in three subsequent trials using the same 31 kDa antigen as an immunising agent (Morton et al. 1995). This molecule was likely to be galectin, which is a beta- galactoside-binding lectin-like

protein (Newton et al. 1997). While these authors did not attempt to test the galectin proteins as vaccine candidates, a galectin (Hco-gal-2) characterised from the gut of *H. contortus* larvae did not confer any protection against infection with *H. contortus* as judged by FEC and worm counts (Newlands et al. 1999).

Surface antigens of *T. circumcincta* L_3 larvae elicit strong bile and serum IgA responses and these antigens were used to vaccinate Finn-Dorset lambs (Wedrychowicz et al. 1992). Worm burdens of immunised lambs were significantly lower than those in challenged control animals (Wedrychowicz et al. 1995). Mucosal and bile IgM antibodies recognising the L_3 surface antigens were more prevalent in the vaccinated lambs, while there were no differences between groups in the levels of mucosal and bile IgA (Wedrychowicz et al. 1995).

There have been many other studies recently that have used components from larval excretions/secretions and particularly gut and surfaces derived proteins to immunise animals prior to challenge. Vaccines targeted against gut antigens are particularly appealing since it has been shown that species of *Haemonchus* and *Teladorsagia* appeared to ingest host antibody (Murray and Smith 1994). The majority of these studies have been conducted with *H. contortus* (Coyne and Brake 2001), and have demonstrated levels of protection ranging from 17 to 95%.

Perhaps the most promising reports are those on the development of a vaccine to *H. contortus* using the hidden antigen approach, where antibodies are raised to a gut antigen not normally exposed to the host immune system (Munn et al. 1993). The *H. contortus* vaccine gives protection of greater than 90% by hyperimmunising sheep with integral membrane proteins extracted from intestines dissected from adult worms (Smith 1993; Tavernor et al. 1992). Recombinant glycoproteins have been produced (Munn et al. 1993). The hidden antigen

approach works very well in protecting against haemonchosis because *H. contortus* is a blood feeder and delivery of parenterally raised antibody is direct. There is some optimism that a similar approach could be used to vaccinate against *T. circumcincta* or *T. colubriformis* because they ingest host immunoglobulin (Murray and Smith 1994). However, which isotype they ingest, and whether or not it will be possible for vaccines to produce that isotype at the mucosal surface is not known.

Barger (1996) wrote that "the most probable time to commercial availability of a vaccine against *H. contortus* based on concealed antigen was judged to be 5 years, and in excess of 10 years for vaccines against other nematode parasites". Five years has now come and gone and there are no commercial vaccines available. Although a considerable amount of research has been conducted since 1996, it is highly unlikely that a vaccine will be available any time soon. Until a vaccine can be demonstrated to produce adequate protection especially in young vulnerable animals, the necessity to use other methods of control, in particular anthelmintics will remain.

1.2.4 Biological control

Two means of biological control that have provoked interest are the use of nematophagous fungi and species of grass that prevent nematode infection (Waller and Faedo 1996).

When the chlamydospores of nematophagous fungi are ingested by sheep and excreted in the faeces they develop and produce hyphae that trap and kill developing nematode larvae (Waller and Faedo 1996). *Duddingtonia flagrans* appears to survive passage through the ruminant gut more efficiently than other nematophagous fungi (Faedo et al. 1998). In addition, *D. flagrans* has been demonstrated to significantly reduce faecal egg counts and increase live weight gain in young sheep on pasture receiving a mixed infection and fed on

barley grains on which *D. flagrans* had been cultured (Knox and Faedo 2001). Similar results were obtained in calves under similar conditions (Sarkunas et al. 2000). Fungal spores present in faecal matter have been demonstrated to reduce significantly the number of eggs and larvae of *H. contortus* (Pena et al. 2002), *T. circumcincta* (Githigia et al. 1997) and *T. colubriformis* (Faedo et al. 1997) from sheep, as well as major parasites from cattle, horses and pigs (Larsen 2000) and most recent work has focussed on this fungus. Clinical disease has been averted in trials where calves naturally infected with *O. ostertagi* were given *D. flagrans* (Nansen et al. 1995). When lambs infected with predominantly *T. circumcincta* were fed *D. flagrans* there was a reduction in newly acquired worm burdens of 62% (Githigia et al. 1997). More work in this field is necessary to determine the optimum dose of chlamydospores and the environmental impact of seeding pasture with nematode trapping fungi.

D. flagrans and other biological methods have potential, alone or in conjunction with other control methods, for the control of nematode infection. Additional research must be conducted to identify the most appropriate method of administration of the biological control agents. Any possible implications to the host and those subsequently consuming or using the animals in question must also be investigated.

Changing the type of feeding can affect the worm burdens and faecal egg counts of lambs suffering from parasitic gastroenteritis, for example, feeding lambs Sulla (*Hedysarum coronarium*) can reduce worm burdens and faecal egg counts (Niezen et al. 1998a;Niezen et al. 1998b). These plants contain condensed tannins, which can reduce larval establishment and increase nematode mortality (Niezen et al. 1998b).

1.2.5 Selection of resistant animals

Genetic resistance is the term usually used to describe instances where particular individuals or breeds show significantly enhanced levels of resistance compared with the mean response of the population (Stear and Wakelin 1998). Genetic resistance to parasites can be studied at a number of levels. In domestic animals, resistance may be evident clinically. For example, when animals are kept in environments known to carry the infective stages of a parasite, variation between individuals may be seen in different patterns of disease, in time of recovery or in differential mortality. Variation at this level may reflect differences in the abilities of animals to control infection, resistance or to tolerate the physiological disturbances caused by infection (Bisset and Morris 1996).

Genetic resistance can be exploited in several ways. Breeds resistant to infection can be used in the place of those that are more susceptible. Substitution of susceptible breeds with resistant breeds is an approach that is practised in the developing world (Baker 1995). For example the Red Maasai breed is more resistant to *H. contortus* than European breeds (Mugambi 1994). However, for breed substitution to be beneficial the new resistant breed must be at least as productive as the breed it replaces. Resistant breeds are often smaller and a common perception is that they are less productive than the larger, more susceptible breeds. Related to this is the misconception of farmers that the new breed is inferior to the larger breed even when evidence to the contrary is available. For breed substitution to be effective, the farmers need to be persuaded, which can be a difficult task to achieve.

Resistant breeds can also be exploited by the use of cross-breeding and the development of a composite population (Nicholas 1987). Most of the references to the use of genetic resistance to infection have been to selection from within populations of sheep for breeding programmes. Such programmes depend upon several factors. They include the heritability of

resistance, intensity of selection, the genetic variation of the trait within the population, the accuracy of the selection process, the time from one generation to the next, and the size of the population (Stear and Wakelin 1998).

Within breed variation is well documented and nematode infection in ruminants is known to have a negative binomial distribution (Barger 1985), with a small proportion of the hosts carrying a large proportion of the parasites. Greater resistance to gastointestinal nematode infection is often associated with greater antibody responses, higher levels of T cell proliferation and increased inflammatory responses, particularly those involving eosinophils and mast cells (Dineen et al. 1978;Douch et al. 1986;Douch et al. 1995a;Douch et al. 1996;Stear et al. 2002b) see section 1.3.

The immune response to nematodes in mature sheep involves an immediate hypersensitivity reaction. The effect of these responses will vary according to the nematode species involved in the infection (Stear et al. 1995c;Stear et al. 1999b). Albers et al. (1987) demonstrated a strong association between resistance to *H. contortus* and resilience, which can be defined as 'the ability of an animal to withstand the pathogenic effects of roundworm challenge and thus maintain acceptable health and productivity with minimal recourse to anthelmintic treatment'. Interest in selecting for resilience was bought about through observations that although some animals had high levels of faecal egg counts, growth levels remained higher when compared with less productive animals with lower egg output (Bisset et al. 2001).

1.3 Immunity to gastrointestinal parasites in sheep

There are three components of immunity to gastrointestinal parasites: decreased worm growth and fecundity, increased percentage of parasite inhibition and decreased worm number. The control of worm numbers can be achieved by preventing the establishment of incoming larvae

(Miller et al. 1983), by the expulsion of developing larvae and ultimately by the rejection of established adult worms (Emery et al. 1993;Miller 1984). Although the initiation of immune responses is antigen specific, the mechanisms underlying worm expulsion can have non-specific effects on other nematodes present in the same or distal part of the alimentary tract (Dineen et al. 1977).

It is clear that the immune response to gastrointestinal parasites in ruminants is T cell dependent. Sheep immune to *H. contortus* can have their immune response partially abrogated after treatment with anti-ovine CD4+ monoclonal antibody (Gill et al. 1993). No comparable effect is seen when the sheep are similarly depleted of CD8+ cells. However, there remains little information on the process of antigen presentation, cellular recruitment and the role of regulatory cytokines in the immune responses in ruminants. It is generally assumed that the effector immune responses in ruminants follow a Th2 type response as seen in rodent models. This is supported by typical Th2 type responses such as eosinophilia, mastocytosis and IgE production in helminth infected sheep (Miller 1984).

Blood and tissue eosinophilia is a typical characteristic of helminth infections (Rothwell 1989). The function of eosinophilia in mucosal parasites remains unclear. In mice their generation in the bone marrow is IL-5 dependent and they have been associated with protection against parasites migrating through tissues (Rothwell 1989;(Doligalska et al. 1999). However, there remains no conclusive evidence that they are required for protection against gastrointestinal helminths (Miller 1996), although they have been associated with some of the detrimental effects of parasitism (Balic et al. 2000). There is an association between the levels of infiltration of eosinophilia and diarrhoea in *T. colubriformis* infected sheep (Larsen et al. 1994).

Mast cells have often been implicated as an effector mechanism in the control of helminth burdens (Huntley et al. 1987;Stear et al. 1995c). The number of *T. circumcincta* parasites is associated with numbers of globule leukocytes in animals over six month of age. In contrast, worm burden is not associated with parasite specific IgM or IgG, numbers of peripheral eosinophils or the numbers of mucosal mast cells (Seaton et al. 1989;Stear et al. 1995c). As globule leucocytes are generally regarded as discharged mast cells (Murray et al. 1968), it would appear that control of *T. circumcincta* worm burdens can be mediated through an immediate hypersensitivity response involving the discharge of mast cells. Although similar accumulations of mast cells have been associated with resistance of sheep to *H. contortus*, (Amarante et al. 1999) there is no unequivocal evidence of a role for mast cells in immune exclusion (Huntley et al. 1992).

Mucosal mast cells could affect parasite loads in any of three ways. Firstly, they may be directly anti-parasitic. They can release a variety of low molecular weight mediators after attachment of parasite molecules to IgE. These mediators may have direct detrimental effects on the parasites survival. Secondly, chymases released systemically and into the gut lumen increase gut permeability, which would allow the leakage of plasma antibody into the gut lumen (Jones et al. 1994;Scudamore et al. 1995). Thirdly, mast cells may have an important role in the coordination of local immune responses through the production of cytokines such as IL-4, IL-5, and IL-6 (Miller 1996).

Antibodies or immunoglobulins are a group of glycoproteins present in the serum and tissue fluids of all higher vertebrates, and on the surface of B cells, where they recognise antigen. There are five distinct classes of immunoglobulin, namely IgG, IgA, IgM, IgD and IgE, although there is no evidence that sheep express IgD (Hein 1998). The different immunoglobulins differ in size, charge, amino acid composition and carbohydrate content. In

addition to the differences between classes, the immunoglobulins within each class are also very heterogeneous. Each immunoglobulin molecule is bifunctional, one region of the molecule is concerned with binding to antigen while a different region mediates effector functions such as binding of the immunoglobulin to host tissues, to various cells of the immune system and to phagocytic cells (Roitt et al. 2001).

The basic structure of all immunoglobulin molecules is a unit consisting of two identical light polypeptide chains and two identical heavy polypeptide chains that are linked together with disulphide bonds in a classical 'Y' shape. The class and subclass of the immunoglobulins are determined by their heavy chain type. In humans there are four classes of IgG, named IgG1 to IgG4. There are also two classes of human IgA but as yet only one class each of IgM, IgD and IgE have been identified (Roitt et al. 2001). Sheep have only two classes of IgG; IgG1 and IgG2, but these are not directly comparable to the IgG classes of other species (Hein 1998). Sheep also have one class each of IgA, IgM and IgE (Hein, 1998). In general, the IgG subclasses are the most prominent isotypes in serum, while IgA is most prominent at mucosal surfaces such as the lungs and gastrointestinal tract, but in ruminants there is a significant amount of IgG1 found in the mucosa.

IgG1 is regarded as a good marker for resistance to *T. colubriformis* in sheep (Douch et al. 1996). However, in cattle infected with *O. ostertagi*, rising IgG1 titres were not associated with immunity (Hilderson et al. 1995). Interestingly, there was a negative correlation between IgA and IgG1 responses in sheep to *T. circumcincta* (Sinski et al. 1995a). IgA may have a role in resistance to this parasite in lambs (Stear et al. 1995b). Therefore, the negative relationship between IgA and IgG1 in animals infected with *T. circumcincta* would cast doubt on whether IgG1 plays a role in immunity to *T. circumcincta,* at least in lambs.

IgE has been associated with developing immunity to *H. contortus* (Kooyman et al. 1997). In this experiment, increased total serum IgE and IgE directed against parasite execretory/secretory product were correlated with decreased worm burdens. However, there was no IgE response detected against L_3 larvae. This would suggest that immunity involving IgE might not be directed at incoming larvae.

In cattle infected with *O. ostertagi,* the influence of IgE on infection is poorly defined. There is evidence that the level of lymph IgE is negatively correlated with parasite burden (Baker and Gershwin 1993). However, there is conflicting evidence for the relationship between infection level and total serum IgE. A high level of infection resulted in elevated IgE in calves compared to those exposed to lower levels (Miller et al. 1996). In contrast, in a different experiment, higher IgE responses were seen in calves moderately infected compared to calves given higher infection (Baker and Gershwin 1993).

IgA production is increased in lambs selectively bred for resistance to *H. contortus* (Gill et al. 1994). In sheep infected with *T. circumcincta* local parasite-specific IgA was correlated with worm length (Smith et al. 1985). Four experiments were recorded, two involving 4.5 month old lambs and two involving 10 month old lambs. the correlation between mean worm length and peak lymph IgA was 0.96. However, correlation between age groups can be misleading. Older lambs have shorter worms on average than younger lambs. Any parameter that varies with age will give a correlation with worm length (Stear et al. 1996b).

During lactation, resistance to helminth infections is often poorer than in non lactating ewes. However sheep infected with *T. circumcincta* have increased levels of IgA in gastric lymph during lactation (Smith et al. 1983). This could suggest that the depression in immunity seen at this time is not due to decreased IgA production. However, the increased level of IgA in the

lymph could also reflect the increased transport of IgA from the gastric mucosa to the mammary gland.

Anti-parasite IgA may interfere with feeding by the parasite (Stear et al. 1995b). IgA may also work indirectly by binding to eosinophils in the mucosa, provoking the release of cytokines. In human infections, IgA/antigen complexes induce eosinophils to release IL-5 (Dubucquoi et al. 1994). Thus, IgA could act indirectly in the orchestration of immune responses at mucosal surfaces.

1.4 Factors affecting the establishment of infection

1.4.1 Sex

In vertebrates the sex of the host influences the infection rate, the intensity of infection and the rate of development of resistance to parasitic infection (Poulin 1996). Male animals are usually more susceptible to infection and develop resistance less quickly than females (Zuk and McKean 1996). Entire (uncastrated) male sheep were found to be more susceptible to infection with *O. columbianum* than entire females (Dobson 1964). Castration of females but not males reduced this sex difference. Other studies have confirmed that castration of males does increase resistance to infection (Barger 1993). These sex differences can theoretically be attributed to several factors. There could differences in physiology between the sexes, differences in behaviour, differences in farm animal management or differences in the rate of ingestion of parasites (Poulin 1996).

Four possible physiological mechanisms have been proposed to explain sex differences in susceptibility to parasitism. They are the deleterious effect in males of being the heterogametic sex, the effect of stress, the direct effect of sex steroids on the parasite, or indirect effects of steroid hormones on the immune system. The heterogamatic hypothesis is

based on the supposition that deleterious recessive alleles normally masked in the homogametic sex would have an effect in males because only one of their sex chromosomes is fully functional. However, in birds where females are heterogametic, femal birds are more resistance to infection than male (Poulin 1996).

There is increasing evidence that male sheep with gastrointestinal nematodes infection show generally greater intensity and high prevalence than females. This has been demonstrated in natural, predominantly *T. circumcincta* infections (Gulland and Fox 1992;Stear et al. 1995b) experimental infections with *H. contortus* (Adams 1989), deliberate infection with *T. colubriformis* (Windon and Dineen 1981) and with *O. columbianum* (Dobson 1964).

These differences are usually attributed to a physiological cause, usually hormonal in origin, as in some of the studies cited above, there may be an association between testesterone and the immune system (Barger 1993). It must be noted that the majority of male sheep in the farming industry are castrated and therefore, sex hormones could not be the main cause of differences between males and females. The more ecological view usually postulated that gender differences in parasitic infection were due to differences in the life histories of males and females, with one sex perhaps eating more or different food, and thus ingesting more infective stages, or perhaps inhabiting an area with a greater tendency to harbour parasites, such as a stream margin (Herd et al. 1992).

In summary, sex differences in susceptibility and resistance to parasite infection are clear but the mechanisms underlying these differences are unclear.

1.4.2 Host age

Age influences the ability of animals to mount effective immune responses to a wide variety of pathogens. For example ruminants less than six months of age are more susceptible than older animals to viral, bacterial and parasitic intestinal and respiratory pathogens (Colditz et al. 1996). The possible reasons for this include not having been previously exposed to the agent to develop active immunity, the suppressive effects of passively acquired maternal antibody or stress associated with early life such as weaning. However, when these factors are taken into account there still appears to be a constitutive immunological hyporesponsiveness to infection (Watson and Gill 1991).

Young lambs have significantly lower proportions of CD4+ and CD8+ lymphocytes but greater proportions of B cells and T19+ lymphocytes (Watson et al. 1994). Sheep less than a year old mount significantly poorer antibody and T cell responses to various antigens and mitogens in comparison to older sheep (Watson et al. 1994; Watson and Gill 1991). However, young lambs are able to mount sufficient immune responses to a variety of antigens to confer immunity. Work on mouse models has challenged the widely held view that neonates are immunologically privileged. They are able to generate immunity provided that antigen is correctly presented to T cells (Forsthuber et al. 1996). This would suggest that the hyporesponsiveness seen in young animals might not result from an incompetent immune system but rather from a lack of adult numbers of immune cells.

1.4.3 Host Nutrition and Growth rate

The importance of nutrition in gastrointestinal nematode infections has been recognised for many decades by veterinarians and health workers who have observed that malnutrition (poor nutrition) and intestinal parasitism share a similar geographical distribution, with the same individuals experiencing both disease states simultaneously (Koski and Scott 2001). It has

been demonstrated on numerous occasions that infection leads to malnutrition and alternatively malnutrition increases susceptibility to infection (van Houtert and Sykes 1996)

In parasitic gastroenteritis there is an increased loss of endogenous protein into the gastrointestinal tract, an increased turnover of epithelial cells, and increased mucoprotein secretion (Parkins and Holmes 1989). Most of this protein in sheep infected with *H. contortus* will be reabsorbed in the gastrointestinal tract though partly as non-protein nitrogen (Rowe et al. 1988) There is however an energy cost to the host in recycling endogenous protein and the gross efficiency of use of metabolisable energy is decreased (Sykes and Coop 1977).

In cattle infected with *O. ostertagi*, gastrin levels increase at the same time as pH, while in sheep infected with *T. circumcincta,* gastrin levels become elevated before abomasal pH, and the elevated pH occurs when the sheep are infected with larval and adult stages (Anderson et al. 1985). This suggests that elevated pH in sheep is not simply a consequence of loss of parietal cell function due to L_4 larvae occupying gastric glands nor that elevated gastrin levels are a consequence of increased abomasal pH.

Anorexia (loss of appetite) is the major consequence of infection for host nutrition (Sykes and Coop 1977;Symons 1985), although the mechanisms remain unknown. Cattle infected with *O. ostertagi* show a relationship between inappetence and elevated gastrin concentration (Fox et al. 1989a). Omeprazole® (human gastric acid secretion inhibitor) inhibits gastric acid secretion and therefore increases blood gastrin (Fox et al. 1989b). Gastrointestinal parasitism in ruminants leads to impairment of live-weight gain, soft tissue deposition, skeletal growth, and milk and wool production (Coop and Holmes 1996).

One example illustrates the influence of gastrointestinal nematode infection on weight gain (Coop et al. 1982).12-week period, animals were kept inside and fed a defined diet. Uninfected animals gained approximately 15 kg, animals infected daily with 1,000, 3,000 or 5,000 larvae gained 13, 11 and 8 kg, respectively. Interestingly, regular anthelmintic treatments of animals receiving 5,000 larvae daily restored weight gain by only 10 kg. While the details differ among experiments the general principles are widely held. Increasing levels of infection reduces weight gain and anthelmintic treatment restores part of the lost weight gain but not to the values seen in uninfected animals (Coop et al. 1982).

An alternative means of assessing the importance of infection on weight gain under natural conditions is to estimate genetic correlations between weight gain or body weight and a trait that indicates infection status such as faecal egg count. The genetic correlation describes relationships between level of infection and weight gain at the individual animal level. Five such studies with predominantly *T. circumcincta* infection have been carried out. All studies estimated the genetic correlation between bodyweight and faecal egg count during natural exposure.

One study in Scottish Blackface sheep estimated the genetic correlation at -0.8 (Bishop et al. 1996); a second in Polish Longwool sheep estimated the correlation at -0.6 (Bouix et al. 1998); a third in Scottish Blackface sheep estimated the correlation at -0.3 (Bishop and Stear 2000a) and a fourth in Scottish Texel sheep estimated the correlation at -0.1 (Bishop and Stear 2001a). A fifth study in feral sheep also produced a negative genetic correlation between egg count and body size (Coltman et al. 2001a) Whilst these correlations do apparently differ, modelling studies suggest that the size of the correlation will vary with the intensity of infection (Bishop and Stear 1999). However, all these relationships are favourable. Together, they indicate that animals with lower egg counts grow more quickly and that variation among

animals in resistance to predominantly *T. circumcincta* infection plays an important role in growth under conditions of natural challenge.

Somewhat surprisingly, the favourable genetic correlations of parasite resistance and growth found for predominantly *T. circumcincta* infections in Europe do not appear for predominantly *H. contortus* or *T. colubriformis* infections in Australia and New Zealand (Albers et al. 1987;Bisset et al. 1992;Douch et al. 1995b;Eady et al. 1998;McEwan et al. 1995). Here the correlations tend to be neutral or unfavourable. The genetic correlations of wool production with parasite resistance are also unfavourable; as egg counts decrease wool production also decreases (McEwan et al. 1995). The unfavourable genetic correlations of wool growth with resistance have been attributed to competition between wool producing cells and cells of the immune system for cysteine (Miller et al. 1998). The unfavourable correlations with growth could reflect differences in management conditions, host breed or parasite species. However, they do suggest that under some circumstances the immune response to gastrointestinal nematodes can have deleterious consequences, this is considered later.

The influence of infection with *T. circumcincta* on growth rate also depends upon the nutritional status of the host (Coop et al. 1995;Stear et al. 2000). As stated earlier, well-fed animals show very few, if any, clinical signs compared to animals on a normal diet (Coop and Kyriazakis 1999). In addition, there may be compensatory protein absorption in the small intestine but this will be affected by infection with small intestinal nematodes (Parkins and Holmes 1989). Mixed nematode infections are likely to be more pathogenic than single species infections for this reason.

Nematode infection can also adversely affect wool production, carcass composition and milk production (Parkins and Holmes 1989). Perhaps the safest conclusion is that nematode infection can decrease production but the severity of production losses varies with the intensity of infection, host nutrition, host immunity and the species composition of the infection (Sykes 1994).

1.5 Phenotypic markers

In order to identify animals with improved resistance to GI parasites a quick, cheap and reliable measurement on the live animal is required. A number of immunological and physiological parameters have been examined as phenotypic or predictive markers of resistance. These have included faecal egg counts (FEC), IgA activity eosinophils, pepsinogen and fructosamine.

1.5.1 FEC

Selection for reduced faecal egg counts is relatively easy to assess and is now part of breeding programmes. Breeding for reduced FEC also has the added benefit in that pasture contamination will be reduced in subsequent generations. However, there remains some disagreement about the effectiveness of using FEC as a marker since they have shown unfavourable associations with production traits. For example, (Bisset and Morris 1996) reported that although genetically low FEC Romney lambs had significantly reduced burdens of the most important nematode species, they had no significant production advantages over their higher FEC counterparts when grazed together under the same larval challenge. Indeed some results have indicated a slightly unfavourable association between FEC and wool production and growth rate in lambs (McEwan et al. 1995). Other limitations to the usefulness of FEC include potential losses of production while drench is withheld for FEC testing, the inability to store samples for long periods and the labour-intensive nature of the method

making automation unlikely (Douch et al. 1996). (Bisset and Morris 1996) suggested that the main benefits of selectively breeding for low FEC are likely to be derived indirectly as a result of reduced pasture contamination as described previously.

1.5.2 Serum pepsinogen

Serum pepsinogen concentrations are an indicator of nematode infection (Reid and Armour 1975). There are a number of factors that lead to increased serum pepsinogen concentrations following infection. The increased pH caused by infection decreases the pepsinogen: pepsin conversion rate which augments the concentration of pepsinogen, the disruption of the epithelial cells allows pepsinogen to leak into the bloodstream. In addition, increases in pepsinogen levels may be caused by intestinal storage cells, called chief cells, no longer storing pepsinogen but releasing it upon production (Stear et al. 1999a).

1.5.3 Serum eosinophil

Peripheral blood eosinophils are thought to be involved with the killing of infective larvae during an infection. They possess extremely potent mediators that can damage and kill nematode parasites. Increases in the number of eosinophils have long been recognised as a characteristic feature of nematode infection. (Stear et al. 2002b) suggests that in the Scottish Blackface, serum eosinophil numbers can be used as an indicator of resistance to *T. circumcincta* infection. In Merino sheep, however, the use of eosinophils as indicators of resistance is not recommended ((Woolaston and Baker 1996).

1.5.4 Fructosamine

Nematode infection induces protein deficiency in animals. Plasma fructosamine concentrations reflect the protein status of an animal and thus are potentially useful indicators of the severity of infection (Stear et al. 2001b).

1.5.5 IgA activity

Resistant animals not only produce more IgA than susceptible sheep but they also produce IgA against specific parasite molecules not recognised by their susceptible counterparts (Stear et al. 1999b). The association between worm burden and local IgA is so strong that it has been suggested that IgA may be more useful than FEC as a marker of susceptibility to infection (Stear et al. 1999b).

1.6 Genetic markers

The problem with all of the phenotypic markers described is the necessity for an infection to establish, at least in part, before the trait can be measured and superior animals identified. It is desirable that selection criteria be developed that will be informative in the healthy animal and this has become a reality with the use of modern technologies utilising genetic markers.

1.6.1 Haemoglobin

Sheep have two major alleles (A and B) for haemoglobin. Several studies have suggested that animals with haemoglobin type AA (HbAA) are more resistant then HbAB, which in turn are more resistant than HbBB, to infection with either *H. contortus* (Preston and Allonby 1979) and *T. circumcincta* (Altaif and Dargie 1978). However, other workers have been unable to confirm this association (Kassai et al. 1990). Therefore, no general conclusion can be drawn concerning the usefulness of haemoglobin type as a predictive marker for resistance.

1.6.2 Interferon gamma (IFN-γ)

An association of an allele at a microsatellite locus located in the first intron of the interferon gamma gene with resistance to gastrointestinal parasitism has been found (Crawford et al. 1997). Confirmation came from a study that reported reduced FEC were associated with

differences at the IFN-γ microsatellite allele in free living Soay sheep infected predominantly with *T. circumcincta* (Coltman et al. 2001a). The same allele was also associated with increased *T. circumcincta*-specific IgA levels. These studies are consistent with the idea that a functional polymorphism leading to reduced expression or efficacy of IFN-γ could enhance the immune response to gastrointestinal nematodes by favouring the activity of the TH_2 cell subset and antibody associated immune mechanisms.

1.6.3 MHC

The major histocompatibility complex (MHC) plays a vital role in host defence against infection, as described previously. The class II genes are among the most polymorphic genes in humans (Bodmer et al. 1990), and assuming the same is true in sheep, makes the MHC an ideal candidate for selection. Associations between the MHC and nematode infection have been reported in several species (Stear et al. 1990).

The relationship between ovine lymphocyte antigens (MHC class I antigens) and parasitological parameters in two flocks of Romney sheep in New Zealand has been investigated (Douch and Outteridge 1989). The study showed that animals that possessed the OLA combination SY 1a + 1b had significantly lower FEC than animals that did not possess this combination. SY 6 occurred significantly more frequently in above average FEC sheep and was associated with significantly higher FEC during secondary challenge infection. Interestingly, OLA SY 6 is also associated with resistance to footrot (Outteridge et al. 1989), and this may be one reason why this method is not yet in use as a selection strategy.

The association of different MHC class II genes has been reported in Scottish Blackface lambs naturally infected, predominantly with *T. circumcincta* (Schwaiger et al. 1995). This study demonstrated that substitution of the most common allele (I) with allele G2 resulted in a

58-fold reduction in FEC in 6 month old lambs and a 22-fold reduction in 5 month old lambs. The MHC class I antigen G13br was associated with reduced FEC in the same flock of lambs (Stear et al. 1996a). Additionally, there was a significant correlation between presence of the G13br class I antigen and *DRB1* G2 allele, or in other words, the alleles are in linkage disequilibrium. Similar associations between alleles at the *DRB1* locus and FEC have been found in Soay sheep (Paterson et al. 1998).

While research continues in many polymorphic genes of the MHC class I and II, the importance of the *DRB1* gene to nematode resistance is highlighted by Schwaiger et al. (1995) and accordingly will receive most attention in this review. The *DRB1* locus within the MHC accounted for approximately 10% of the total variation in FEC, and with the addition of sex, sire and dam, the model accounted for 81% of the variation in FEC in 6 month old lambs (Schwaiger et al. 1995).

The direct involvement of *DR* molecules in regulating resistance against gastrointestinal nematodes is likely because of their central role in antigen presentation and antibody responsiveness (Schwaiger et al. 1995). The substitution of the I for the G2 allele by selective breeding should produce animals with superior resistance to *T. circumcincta*. However, associations with other economically important traits must first be checked in case breeding for the G2 allele selects animals with poorer productivity and/or increased susceptibility to other pathogens.

1.7 Objectives of this study

Gastrointestinal nematodes are responsible for morbidity and mortality in many sheep rearing enterprises worldwide, particularly in the tropics. In the temperate UK, infection is a major

contributor to reduced production particularly through sub-clinical infections (Armour and Coop 1991).

Although nematode infection can be controlled by the use of anthelmintics and grazing management, the rapid evolution of resistance to drugs (Jackson 1993) and the impracticability of control through management has created an urgent and increasing need to develop alternative strategies to control nematode infection. These include the use of genetically resistant sheep (Stear and Murray 1994), improved dietary supplements (Holmes 1993), the use of nemtophagous fungi (Waller 2003) and the development of vaccines (Smith 1999). The applicability of all these methods will be greatly helpful with better understanding of the interaction between the parasite and host in controlling nematode infection

The general purpose of this thesis was to investigate several phenotypic and genetic markers for resistance to nematode infection in naturally infected Scottish Blackface sheep. The phenotypic markers were faecal egg count and IgA activity against third-stage larvae. The genetic marker was the class II region of the major histocompatibility complex.

The specific objectives were to:

i. Investigate the variation in faecal egg count distribution in Scottish Blackface sheep naturally infected with *T. circumcincta* in August, September and October for three consecutive years from 2001 to 2003..
ii. To investigate the variation in faecal egg counts among populations of sheep from two farms in central and southern Scotland.
iii. To explore whether IgA activity against L3 could be used as an indicator trait for resistance to nematode infection.

iv. To determine the relationship between IgA activity against L3, faecal egg counts and growth rate.

v. To search for QTLs associated with nematode parasite resistance in Scottish Blackface sheep using faecal egg counts and IgA activity as indicator traits.

CHAPTER TWO

GENERAL MATERIALS AND METHODS

In this chapter are detailed the materials and methods used in this study. Details of specific assays and any minor changes to these methods are detailed in the relevant chapters. Assistance with methods is acknowledged in the acknowledgement section.

2.1 PARASITOLOGY

2.1.1 Faecal Worm Egg Count

Faeces samples were taken directly from the rectum of the lambs and stored at 4°C until processed. A modified McMaster salt flotation technique (Miller and Nawa 1979;Wells 1963) was performed to estimate the concentration of nematode eggs in the faeces. Three grams of faeces were added to 42 ml of tap water, the mixture was then homogenised mechanically and poured through a 250-micron aperture sieve and the filtrate was transferred to a 15 ml glass test tube and spun for two minutes at 560g. The supernatant was discarded and the faecal pellet was broken up by vortexing and the tube was filled to its former level with saturated sodium chloride solution. The tube was gently inverted six times, until the suspension was mixed. Both chambers of a McMaster egg counting slide (Gelman Hawksley Ltd., Northampton, England) were filled using a plastic Pasteur pipette. The preparation was then examined using the x25 objective of a Stereomicroscope. The number of eggs present in both chambers was counted and the result was multiplied by 50 to give the number of eggs per gram. To improve accuracy of the technique, four slides were counted per animal.

2.1.2 Herbage Analysis

The importance of pasture larval counts is to determine and identify the differences in larval species and concentration of infective larvae L_3 of parasitic nematodes on pasture. A w-shaped route across the paddock (diagonally four times) was used to collect herbage samples (Taylor 1939). The samples were collected by hand, where fifty evenly spaced stops were made along each route. At each stop, four plucks of grass were taken to make a total of 200

plucks per plot. Herbage samples were collected in a plastic bag, which was weighed and put into a small hand operated washing machine (Easy Pressure Washer, Classic Supplies Ltd., Leeds). Eight litres of lukewarm water were added and the bag was tied and the machine turned through two hundred revolutions. The herbage was filtered through a 2mm mesh sieve into a bucket. The washed grass was then spread on a tray and dried in an incubator at 70°C. When the grass was completely dried, the herbage was weighed again. The dry weight was then used in the final calculation of numbers of larvae per kilogram-dried herbage (L3/kdh). A 38-micron sieve was used to filter the washings contained in the bucket where the material was retained. The larval suspension was drawn through a coarse filter paper (Whatmans Grade 113, 18.5cm) using a Buchner funnel and vacuum pump. A single milk filter paper (Maxa Milk filters, A. McCaskie, Stirling) was put on top of the retained material, the combination inverted and placed on a Baermann filter funnel filled with lukewarm water. After a minimum of six hours, 10 ml of fluid was withdrawn and the larvae were differentiated and counted.

2.1.3 Weight of the lambs

Individual body weight of the experimental lambs were recorded using a sheep weighing scale at each blood sampling and faecal collection dates during October 2001, 2002 and 2003.

2.1.4 Preparation of third stage larvae

The strain of *T. circumcincta* larvae used was a gift of the Moredum Institute, Pentlands Science Park, Penicuik, Scotland. Sufficient numbers of larvae for the preparation of somatic extracts were obtained by passage of the larvae through parasite-naïve sheep at Glasgow University Veterinary School.

Third-stage larvae were collected from faecal cultures of eggs of deliberately infected sheep and exsheathed in 1% Sodium hydrochloride (Milton 2, Richardson-Vicks Ltd) in phosphate buffered saline (PBS, pH 7.4) for 10 minutes at 37°C. Exsheathed larvae were then resuspended in 50 ml PBS and centrifuged at 100g for 10 minutes. The supernatant was removed and then washed twice. The larval pellet was washed once in PBS containing 100IU ml^{-1} penicillin, 0.1mg ml^{-1} streptomycin, 2.5µg ml^{-1} amphotericin B and 0.05 mg ml^{-1} gentamicin to prevent any contamination (Sinski et al. 1995a). Larvae were then given a final wash in 50 ml of 10 mM Tris buffer (pH 8.3), containing 1mM disodium ethylene diamine tetracetic acid (EDTA), 1mM ethylene glycol bis (2-aminuteso ethyl ether)-N,N,N',N'-tetracetic acid (EGTA), 1mM N-ethylmaleimide (NEM), 0.1µM pepstatin, 1mM phenyl methyl sulphonyl fluoride (PMSF), and 0.1mM N-tosylamide-L-phenylalanine chlorormethyl ketone (TPCK) as protease inhibitors (protease inhibitor solution) (Sinski et al. 1995a). The larval pellet was then resuspended in an equal volume of protease-inhibitor solution containing 1% sodium deoxycholate and homogenised using a handheld electric homogeniser (Janke & Kunkel IKA Labortechnik, Staufen, Germany) on ice. When larvae were completely homogenised, they were centrifuged at 500g for 20 minutes and the soluble extract was filtered through a sterile 0.2µm syringe filter. The extract was again spun at 500g for 20 minutes and the supernatant aliquoted and stored at –80°C for subsequent use.

2.2 Serological methods

2.2.1 Blood Samples

Blood samples were collected in October 2001, 2002 and 2003 by jugular venepuncture into evacuated glass tubes containing 20 mM disodium EDTA (Becton Dickinson UK Ltd, Oxford, UK) as an anticoagulant. Plasma and buffy coats (the leucocyte rich region of whole blood) were obtained by centrifugation at 1000g for 30 minutes and stored at –20°C until further use.

2.2.2. Preparation of rat anti-sheep IgA monoclonal

The rat hybridoma cell line (M1521) was obtained from Dr. S. Hobbes, Dr. P. Bird and Professor I. McConnell and maintained by the following culture protocol: A vial of frozen cells was removed from liquid nitrogen, thawed quickly in water at 37°C and suspended in 10 ml of culture media (90% RPMI 1640 (+2 mM L-glutamine), 10% foetal calf serum (FCS), and 50 µg/ml gentamicin (Gibco). Cells were pelleted by centrifugation at 150g for 5 minutes. Cell viability was checked by mixing 100 µl cell suspension with 100 µl 0.4% Trypan Blue stain and the number of cells was counted using a haemocytometer. Cells were diluted into culture media to give a cell concentration of $3x10^5$ cells per ml. Cultures were incubated at 37°C in 5% CO_2 and examined on a daily basis.

After 4 days, when the cells were confluent, cultures were centrifuged for 10 minutes at 150g. The supernatant was removed and stored at –20°C in 1 ml aliquots. Cells were then either re-seeded to produce more antibody supernatant or frozen for subsequent culture as follows: cells were resuspended in 10 ml culture media, counted as described previously, then resuspended at a concentration of $5x10^6$/ml in freezing medium (10% dimethyl sulphoxide, 20% FCS, 70% RPMI 1640 +2mM L-glutaminase). Cell suspensions were then frozen in a "Mr Frosty" cell cryopreservation container (Nalgene) at – 80°C for 24 hours which allowed a cooling rate of –1°C/minute. Vials were then transferred to liquid nitrogen for long term storage.

2.2.3 ELISA assays

An Enzyme-linked immunosorbent assay (ELISA) was used to detect ovine IgA activity against somatic larval extracts of third-stage larvae from *T. circumcincta*. Each well of a 96-well flat-bottomed microtitre plate (Nunc) was coated with 100 µl of larval antigen preparation (L_3) at 5µg ml^{-1} in 0.06M sodium carbonate buffer pH 9.6 overnight at 4°C. The

plates were washed five times with 0.01M PBS containing 0.05% Tween 20 (PBS-T). In order to minimise non-specific binding of antibody, the wells were incubated with 200 µl per well of PBS-T containing 4% skimmed milk powder for two hours at 37°C. The plates were then washed five times in PBS-T. The individual sheep plasma samples were diluted 1:10 in PBS + 0.4% skimmed milk (PBS-TSM). The plates were then incubated with 100 µl per well, in duplicate for 30 minutes at 37°C. Positive and negative controls were diluted 1:10 in PBS-TSM and run in triplicate on each plate to minimise the effect of variation between plates on different days. After incubation and another five washes in PBS-T, plates were incubated with 100 µl per well of a monoclonal rat IgG anti-sheep IgA diluted in PBS-TSM for 30 minutes at 37°C. After five washes in PBS-T, the plates were incubated with 100 µl per well of a mouse anti-rat IgG alkaline phosphatase antibody conjugate (Sigma) diluted in PBS-TSM for 30 minutes at 37°C. After a final five washes in PBS-T, the plates were incubated in 100 µl per well with Bluephos® Microwell Phosphatase Substrate system (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA) at 37°C. The optical density of each well was then read at 635 nm with a multichannel spectrophotometer (Titertek Multiscan MC, Labsystems, Oy, Finland or Dynex MRX, Dynex Technologies, Ashford, UK) for 15 minutes. Usually this was done at five minute intervals, until the positive control optical density reading was between 1.5-2. Each batch of monoclonal and secondary antibody was titrated to determine the appropriate concentration. This procedure was carried out on the Grifols Triturus® ETA Analyser.

2.2.4 Optical density indices

Optical density is the absorbance of an optical element for a given wavelength λ per unit distance. The Positive and negative controls in each ELISA assay were pooled plasma samples from individual animals which had given either very strong (positive control) or very weak (negative control) optical densities using the methods of Sinski et al. (1995). To

minimise the variation between results obtained on different days and between plates, optical densities for each sample were transformed into an optical density (OD) index using the following equation (Sinski et al. 1995a):

$$OD\ Index = \frac{OD\ of\ test\ sample - OD\ of\ negative\ control}{OD\ of\ positive\ control - OD\ of\ negative\ control}$$

2.3 Molecular Biology

2.3.1 DNA Extraction

2.3.1.1 Solutions and Media

1M Tris-HCl pH7.5

A total of 121g Trizma base (Tris [hydroxymethyl] aminomethane; Sigma-Aldrich Company Ltd, Poole, England), was weighed and dissolved in 800 ml distilled water (dH_2O) and the pH was adjusted to 7.5 using Microprocessor pH meter (HANNA Instrument). The volume was adjusted to 1L and the solution was stored at 4°C.

1M $MgCl_2$

A total of 203.3g $MgCl_2.6II_2O$ (BDH Chemicals Ltd, Poole, England), was weighed and dissolved in 800 ml dH_2O. The volume was adjusted to 1L and autoclaved. The solution was stored at 4°C.

5M NaCl

A total of 292.2g NaCl (Sigma- Aldrich) was weighed and dissolved in 800ml dH_2O. The volume made up to 1L, autoclaved and stored at 4°C.

0.5M Ethylene Diamine Tetra Acetic Acid EDTA pH8.0

A total of 93.05g EDTA (Ethylene Diamine Tetra Acetic Acid- Sigma-Aldrich) was weighed and made up to 500 ml in dH_2O, the pH was adjusted to 8.0, the solution was autoclaved and then stored at 4°C.

10% Sodium Dodecyl Sulphate SDS pH7.2

A total of 100g SDS (Sodium Dodecyl Sulphate-Sigma-Aldrich) was weighed and mixed with 800 ml dH_2O. The solution was heated to dissolve the SDS, and then the pH adjusted to 7.2. The volume was made up to 1L and the solution stored at room temperature.

Proteinase K

A total of 100mg Proteinase K (Sigma-Aldrich) was dissolved in 25 ml dH_2O to give 4 mg proteinase K/ml. 500 µl of this solution was mixed with 500 µl 10% SDS and frozen at -20°C, until required.

3M Sodium Acetate solution pH5.2

A total of 204.1g Sodium Acetate.$3H_2O$ (BDH-Limited) was mixed with 350 ml dH_2O and the pH was adjusted to 5.2 with Glacial Acetic Acid. The volume was made up to 500 ml with dH_2O, autoclaved and stored at 4°C.

TBE buffer

A total of 54g of Tris-borate and 27.5g boric acid were dissolved in 800ml dH_2O, 20ml of 0.5M EDTA pH 8.0 was added and made up to 1L with dH_2O. The solution was autoclaved and stored at 4°C.

Phenol: Chloroform: Isoamyl alcohol (25:24:1)

A total of 250 ml equilibrated phenol (Sigma-Aldrich), 240 ml chloroform (Sigma-Aldrich) and 10 ml isoamylalcohol (Sigma-Aldrich), were mixed together and stored in a dark bottle at 4°C, until required.

Chloroform: Isoamy alcohol (24:1)

A total of 240 ml chloroform (Sigma-Aldrich) was mixed with 10 ml isoamyalcohol (Sigma-Aldrich) and stored at 4°C until required.

Lysis Buffer

A total of 109.536g Sucrose (Sigma-Aldrich) and 10 ml 1M Tris-HCl pH7.5 and 5 ml 1M $MgCl_2$ were added to dH_2O and the volume made up to 990ml. The solution was then autoclaved and 10ml of 10% Triton × 100 (Sigma-Aldrich) was added. The buffer was stored at 4°C.

Digestion Buffer

A total of 7.5ml 5M $NaCl_2$ and 25 ml 0.5M EDTA (pH8.0) was mixed with dH_2O and made up 500 ml. The solution was autoclaved and stored at 4°C, until required.

2.3.1.2. DNA Extraction Procedure

DNA Extraction is a routine procedure to collect DNA for subsequent molecular analysis. DNA was extracted by using the lymphocyte-rich layer on top of the red cells after centrifugation of anti-coagulated blood (buffy coat). Each thawed buffy coat was added to 50 ml cold lysis buffer. The tube content were mixed thoroughly several times by inversion of the tubes. Tubes were then stood on ice for 10 minutes and afterward centrifuged for 10 minutes at 4°C at 800g. The supernatant was discarded and the tubes were blotted on a paper

towel, the pellet was resuspended and mixed in 5 ml Digestion buffer, then the tubes were centrifuged at 800g for 10 minutes at 4°C. The supernatant was discarded and the tubes were blotted on a paper towel. The pellet was resuspended and mixed with 4 ml Digestion buffer. A 1ml Proteinase K solution was added to every sample and incubated in a 56°C water bath for 24 hours. Then the samples were removed from the water bath and 5 ml equilibrated phenol (Sigma-Aldrich) was added and mixed gently to form an emulsion. The samples were left for 10 minutes, and then centrifuged for 10 minutes at 800g. The clear aqueous layer was transferred to 50 ml tubes, where it was remixed gently with phenol: chloroform: isoamyl alcohol (25:24:1) and then centrifuged for 10 minutes at 800g. The clear aqueous layer was collected and transferred to 50 ml tube. The previous step was repeated. The new aqueous layer was transferred to 15 ml plastic tubes where 5ml chloroform: isoamyl alcohol (24:1) was added and mixed. Tubes were centrifuged at 800g for 10 minutes. The clear aqueous layer was removed to fresh 30 ml plastic tubes containing 1 ml 3M sodium acetate and 12.5 ml cold ethanol was added. The tubes were inverted several times until the DNA precipitated. DNA was removed with a heated sealed pipette and rinsed in 500 µl 70% ethanol in a tube, which was allowed to air dry, then placed in a 2ml plastic tube containing 200 µl TE buffer. The tubes were then heated at 56°C in a water bath for 1 hour. Lastly, the tubes were gently shaken for 2-7 days at 4°C to resuspend the DNA.

2.3.2. Cloning of MHC class II DRB1 alleles

2.3.2.1. Solutions and Media

ERB3- primer solution

A total of 20µl of ERB3 at 100 pmol/µl (MWG-Biotech AG) was diluted in sterile distilled water to make up a 20 pmol/µl ERB3.

ERB3 primer 5'- CTCTCTCTGCAGCACATTTCCT

SRB3- primer solution

A total of 20µl of SRB3 at 100 pmol/µl (MWG-Biotech AG) was diluted in 80µl sterile distilled water to make up a 20 pmol/µl SRB3.

SRB3 primer 5'-CGCTGCACAGTGAAACTC

The primers mentioned above were used for preparation of a Master Mix solution (see section 2.3.2.2), to amplify a regon lay in chromosome 20 (DRBI EXON II).

1.5% Agarose gel

A total of 0.750g Seakem® LE Agarose (Cambrex Bioscience, Rockland, Inc. Rockland, ME USA) was mixed with 50 ml TBE buffer in a 200 ml Pyrex beaker on a magnetic stirrer (Stuart Scientific) which was set at a low level. The beaker was then placed in a microwave oven (Zanussi) and heated for 2 minutes at high setting until the TBE and agarose had fully dissolved. The beaker was replaced on the magnetic stirrer at the low level setting to cool down and 5µl ethidium bromide was added to the agarose. When the agarose temperature reached approximately 55°C, it was poured in a levelled gel-casting tray with the coomb removed and any air bubbles which appeared were removed using a needle. At this point the comb was put in the gel-casting tray and the gel allowed to set for 30 minutes in the cold room (4°C) before use.

X-Gal Solution

Atotal of 100mg X-Gal (5-bromo-4-chloro-3-indolyl-β-D- galactoside\ LIFE TECHNOLOGIES™, Paisley, UK) was mixed with 2 ml dimethylformamide (Sigma-Aldrich) and stored at -20°C.

LB agar

A total of 5g LB broth (Lennox L broth, Sigma-Aldrich) and 3.750g Agarose (Seakem® LE Agarose- Cambrex Bioscience Rockland, Inc. Rockland, ME USA) was mixed with 250 ml dH_2O. The solution was autoclaved and allowed to cool in a water bath at 42°C and 125µl of 50µg/ml ampicillin (Segma) was added and mixed properly with the solution. 25 ml of this mixture was poured into sterile Petri-dishes plate in a sterile condition (the quantity of this solution is sufficient for 10 plates).

LB broth

A total of 5g LB broth (Lennox L broth, Sigma-Aldrich) was mixed with 250ml dH_2O and was autoclaved. The solution was left to cool until it reached 55°C when 125µl of 50µg/ml ampicillin (Segma) was added and mixed with the solution. The solution was kept at 4°C until required.

2.3.2.2 Cloning Procedure

Preparation of PCR reaction

PCR products (DRBI EXON2 allele) were prepared using lamb genomic DNA, as described in section 2.3.1.2, Taq polymerase (Qiagen) and Master Mix (MM) solution. PCR master mix was prepared in a PCR hood as shown in Table 2.1.

Table 2.1 MM solutions for MHC Class II DRB1 EXON2 genotyping

Reagent	Concentration	Amount
ERB3	20pmol	1.0µl x n+1
SRB3	20pmol	1.0µl x n+1
DNTPs	10mlmol	1.0µl x n+1
MgCL		2.0µl x n+1
10x BUFFER		5.0µl x n+1
H_2O		39.8µl x n+1
The MM solution was irradiated for five minutes in an ultra violet (UV) box.		
Taq polymerase		0.2µl x n+1
DNA sample		1.0µl per sample
Negative		1.0µl for negative control

A total of 49µl of MM was mixed with 1µl DNA and 49µl MM with 1µl H_2O for the negative control using 0.2ml Eppendorf tubes. Samples were placed in a thermocycler (Gene Amp-PCR system2700 Version2.0- Bio systems A&B). The PCR conditions were as follows:

1 cycle pre-PCR	94°C for 6min
	61°C for 2min
	72°C for 2min
35 cycles	94°C for 1min
	61°C for 2min
	72.0°C for 2min
1 Hold	72°C for 15min
1 Hold	4°C for ∞

The reaction products were analysed by 1.5% agarose gel electrophoresis. The comb was removed from the gel, and the gel was placed in a tank, then 1xTBE buffer was added until the gel was covered by approximately 3mm. For each sample, 3µl PCR product was added and mixed with 1µl gel loading buffer, which was then loaded into a well. Each gel contained a negative control and a 100bp DNA ladder (Invitrogen). The gel was then run at 100v, until the dye font reached the end. The gel was then removed and photographed using a gel documentation system (UVI tec) in order to confirm the presence of the PCR products (DRB1 EXON II).

TOPO® Cloning Reaction

The PCR products were TOPO® (Invitrogen) cloned into the pCR®2.1-TOPO® vector (Table 2.2) and chemically competent *E. coli* cells were transformed with the recombinant vector according to the manufacturer's instruction (TOPO 10 competent cells, Invitrogen).

Table 2.2 TOPO® Cloning Reaction

Reagent	**Reaction Volume**
Fresh PCR product	2.0µl
Salt Solution	1.0µl
Sterile Water	2.0µl
TOPO® Vector	1.0µl
Final Volume	6.0µ

The reaction was mixed gently into 0.5ml microtubes, and incubated at room temperature for 5 mins. The reaction mixture was placed on ice. A 2µl of the cloning reaction was mixed

gently with *E. coli* cells and incubated on ice for 5 mins. The cells were then heat shocked for 30 seconds at 42°C in a water bath. Immediately the tube was placed on ice. A total of 250µl of SOC medium was added to the tube which was tightly capped and rotated horizontally at 200 rpm at 37°C for 1 hour. 20µl of transformation mixture was mixed with 30µl of SOC medium and spread on prewarmed LB-agar plates containing X-Gal and 50µg/ml ampicillin. Plates were incubated overnight at 37°C. Blue and white colonies were observed the following day. A total of 10 white or light blue colonies were isolated and each colony was cultured overnight in 3 ml LB broth containing 50µg/ml ampicillin in universal tubes at 37°C in an orbital shaker (innova 4000, New Brunswick Scientific) at 225 rpm. Plasmid DNA was checked for presence of the correct insertion by PCR amplification as mentioned previously (see section 2.3.2.2).

Purification of plasmid DNA

PCR products were purified following the QIAprep® PCR spin miniprep kit protocol (QIAGEN, UK). Briefly, the DNA adhered to the filter within the column, separating it from all other components of the PCR reaction, which were washed away with various buffer solutions. The plasmid DNA was finally eluted in 50µl Elution buffer (EB) (QIAGEN, UK)

Sample preparation for DNA sequence analysis

Cloned fragments were chemically labelled with fluorescent dyes by cycle sequencing to facilitate the detection and identification of the DNA. Cycle sequencing utilizes successive rounds of denaturation, annealing and extension in a thermocycler to create a linear amplification of extension products. More specifically, PCR reactions were performed using plasmid DNA samples in a total volume of 20µl containing 3µl of primers, 4µl 10x buffer, 7µl sterile water, 2µl DNA and 4µl of Big Dye™ Terminator Cycle sequences ready reaction

(ABI Prism). Samples were prepared in the thermocycler (Gene Amp- PCR system2700 Version2.0- Bio systems A&B), incorporating 25 cycles of amplification as follows:

25 Cycles	96°C for 10seconds
	50°C for 5second
	60°C for 4minutes
	4°C

DNA was then purified by precipitation methods using 16µl of deionised water and 64µl of non-denatured 95% ethanol and kept at room temperature for 15 minutes. Pelleted DNA (200 g for 20 minutes) was washed in 250µl of 70% ethanol and re-pelleted (200 g for 10 minutes) before all ethanol was removed and dried at 90°C for 1 minute. Template suppression reagent (25µl formamide) was then added, and the mixture left at room temperature for 2 minutes. The mixture was then heated to 95°C for 2 minutes and chilled before loading on the ABI PRISM® 3100 Genetic Analyzer.

DNA sequencing

Samples were loaded and run on the ABI PRISM® 3100 Genetic Analyzer (PE Applied Bio systems, UK), under standard sequencing conditions for generation of automated sequence data. The ABI PRISM® 3100 Genetic Analyzer is a multi-colour fluorescence-based DNA analysis system using the proven technology of capillary electrophoresis with 16 capillaries operating in parallel. The Genetic Analyzer is fully automated from sample loading to data analysis.

Sequence evaluation

The output was in the form of a chromatogram and a sequence file. The chromatogram was examined with Chromas software to resolve any ambiguous base assignments. The sequence

file was analysed with the fasta program in genetic Computer Group (GCG) Software to search for similarity between the new sequence and existing DRB1 sequences which were obtained after searching Genbank database and published data. Any sequences which differed from existing sequences were confirmed in at least two separate PCR reactions.

2.4. Statistical analysis

For clarity, the relevant statistical analyses are described in each chapter.

CHAPTER THREE

The distribution of faecal egg counts and pasture larval counts in naturally infected sheep

3.1 Introduction

The number of infective larvae ingested by sheep will depend upon the number of eggs deposited by infected sheep, the rate of development of eggs to larvae, the larval survival rate and the grazing pattern of sheep (Stear et al. 1995b). Faecal egg count is widely used to assess levels of nematode infection. Pasture larval counts have also been used to determine the intensity of challenge (Boag and Thomas 1971;Stear et al. 1995b).

Lambs are born worm free with little innate resistance to parasite infection. Young lambs are exposed to two potential sources on infection, larvae which have overwintered on the pasture and those deposited by ewes during the peri-parturient period (Gibson and Gibson 1973;Leathwick et al. 1995).

As the season progresses, the relative numbers of the different species changes (Michel 1976) and tends to follow a definite trend in dominance. Due to its ability to overwinter (Waller and Thomas 1978), *Teladorsagia* is particularly prevalent in early summer (Reid and Armour 1972) followed by *Trichostrongylus*, *Cooperia* and *Haemonchus* species (Cornwell 1975). Levels of larvae can, however, rapidly increase in response to favourable climatic conditions, and eggs deposited at varying times can sometimes reach the infective stage at the same time (Thomas and Boag 1973), and this leaves animals exposed to potentially overwhelming infection. There tend to be two main waves of infection over a season, the first in late spring and the second in mid-to-late-summer (Thomas and Boag, 1973; Cornwell 1975). The first is derived either from overwintered larvae (Thomas and Boag, 1973) or in combination with eggs deposited during the PPR (Michel, 1976) while the second wave is derived from eggs deposited by lambs from the initial challenge in the spring (Thomas and Boag, 1972).

Stear et al (1995) studied the seasonal pattern of nematode infection in sheep over two consecutive years. The mean values for faecal egg counts at monthly intervals between May and September showed that egg counts increased from May to June in both years but otherwise there was no consistent pattern (Stear et al. 1995b). The same author suggested that lambs which experienced a heavier infection earlier in life were more able to resist subsequent infection, possibly as a consequence of more efficient immunological priming. Similar results were reported by (Gill 1991), with the progeny of resistant sires having higher FECs and worm burden than controls after primary infection with *H. contortus* but lower egg counts and worm burden following secondary infection.

Variation among months in mean faecal nematode egg count was attributed to differences in temperature and moisture from year to year, frequency of anthelmintic treatment and stocking density and movement (Boag and Thomas 1971). Differences in weather patterns may be largely responsible for differences in the infectivity of larvae at different times of the year and also for differences between years (Stear et al. 1996b). In spring, infected ewes often exhibit a peri-parturient rise in the output of parasite eggs and as spring temperatures rise the eggs develop to infective larvae.

The acquisition of new larvae from pasture in the spring is sensitive to changes in environmental conditions especially in the UK where weather conditions can vary considerably both within the season and between years. Infected lambs contaminate the pasture. By late July and August pasture contamination levels rises to become a significant threat to lambs (Gettinby et al. 1989). The number of *T. circumcincta* L3 on herbage increases markedly from mid-summer onwards and this is when disease appears, as the eggs deposited in the first half of the grazing season from April to June give rise to larvae from July to October (Boag and Thomas 1971;Urquhart et al. 1996).

Anthelmintic use has a strong effect in limiting worm burdens and in particular in controlling adult worms. However, eggs have been found in faeces within 14 days after drug treatment (McKellar and Marriner 1987). Also lambs from dosed ewes have shown a substantial rise in faecal egg count in August and September, while the highest faecal egg counts were reported in lambs from undosed ewes in September (Boag and Thomas 1973).

Theoretically, distribution pattern of parasites within host populations can be grouped into three broad categories: underdispersion, random and overdispersion (Sreter et al. 1994). A number of data support the view that the distribution of worm number per host is characteristically overdispersed within both animal and human population. Parasite abundance is measured ultimately by the statistical distribution of parasites between hosts. Parasites are characteristically aggregated in their hosts (Shaw and Dobson 1995), this pattern can generally be described empirically by the negative binomial distribution. Theoretical models have shown that the observed patchiness in parasite abundance can have important consequences for host-parasite population dynamics (Anderson 1978;Grenfell et al. 1995;Roberts et al. 2000;Smith 2000). The distribution of parasites may have important implications also for the estimation of economic losses due to helminth infections in farm animal populations (Barger 1985). The dispersion pattern of parasites is one of the principal factors controlling the population dynaics of helminth infection (Anderson and May 1985). Despite the theoretical significance and the important, practical implications of dispersion patterns of helminth parasites in farm animal populations, the number of parasite frequency distributions documented in the literature is limited

(Barger 1985) reported that the relatively low degree of overdispersion (k = 1.39) may mitigate against the extension of selective treatment programmes. Notwithstanding this, the

degree of aggregation is high enough to form the basis of breeding programmes for higher resistance to gastrointestinal nematodes (Barger 1989).

The purpose of this chapter was to describe changes in the mean and distribution of faecal egg counts and especially the relationship between mean parasite load and aggregation (inversely measured by negative binomial parameter, *k*) for cohorts of hosts, to identify nematode species in the pasture, and also to investigate the seasonal pattern of *T. circumcincta* infection in naturally infected lambs over a three year period.

3.2. Materials and Methods

3.2.1. Animals

A flock of 758 straightbred Scottish Black face lambs was studied from 2001 to 2003. All lambs were from a research farm in East Scotland. Lambs were weaned at four months of age and sampled in August, September and October of 2001 to 2003. Anthelmintic treatment was given at each sampling either ivermectin (Oramec Drench, Merial Animal Health) or levamisole (Nilverm, Schering-Plough Animal Health), which were rotated between years, at the dose rates recommended by the manufacturers, which were 7.5mg kg^{-1} and 5mg kg^{-1} respectively.

3.2.2. Parasitological methods

The parasitological methods are described in Chapter two section 2.1. Briefly faecal egg counts were made according to a modified McMaster method with saturated salt solution. Each sample was counted on four separate McMaster chambers and each egg counted represented 50 eggs per gram. These counts will be referred to as epg1, epg2, epg3 and epg4. The dominant nematode on this farm was *T. circumcincta*.

Herbage samples were collected from different fields; Lambs were moved around from field to another, depends on the field condition. As the field exhausted by grazing lambs, sheep were moved to another well-grazed field. To facilitate further analysis, number have been given to each field in each month that lambs were grazed, but it is not necessarily to be the same field number in the following months or year.

3.2.3. Statistical analyses

Means and ranges were calculated with the means procedure of the SAS statistical package (SAS Institute, Cary, North Carolina, Version 8). Chi-square tests were used to examine the goodness of fit to the negative binomial distribution. The negative binomial distribution has often been used empirically to describe the distribution of parasites among individuals (Anderson & May, 1992). The distribution is defined by its arithmetic mean and a shape parameter k, which is an inverse index of the extent of dispersion; as k decreases toward zero the distribution become more dispersed. The SAS program estimates the parameter k by maximum likelihood (Bliss and Fisher 1953). The relationships among faecal egg counts obtained on different dates were estimated by the correlation coefficient using the correlation procedure on the SAS statistical package. Egg counts were overdispersed and were transformed prior to statistical analysis by taking the logarithm of the egg count plus ten, to produce approximately normally-distributed data. Correlation analysis assumes normally-distributed data.

3.3 Results

Tables 3.1, 3.2 and 3.3 show the geometric mean faecal egg counts, standard error of the mean and range of three successive years of values observed in faecal egg counts in August, September and October. In 2001 table 3.1 shows that the mean faecal egg count was 363.9 in August then rose in September to 408.2, and fell to 278.2 in October. In 2002, the pattern of

faecal egg count differed from 2001 (Table 3.2), in August, the mean was 260.4 then decreased to approximately 200 and fell slightly in October to 181.6. In August 2003, the mean was 150.7, increased to 271.3 in September, and then decreased slightly in October to 252.7 (Table 3.3). From the above, the mean faecal egg counts in August 2003 were slightly less than the two previous years. The range revealed that high counts can occur even in quite young animals (for example in August the maximum epg was 2900).

Table 3.1 Eggs per gram (epg) of faeces in lambs sampled in 2001

Date of sampling	Number of animals	Mean	Std. Error of mean	Minimum	Maximum
August	240	363.9	26.5	0	2900
September	216	408.2	29.6	0	2388
October	229	278.2	19.1	0	1700

Table 3.2 Eggs per gram (epg) of faeces in lambs sampled in 2002

Date of sampling	Number of animals	Mean	Std. Error of mean	Minimum	Maximum
August	248	260.4	26.7	0	3275
September	256	201.1	16.9	0	2550
October	261	181.6	13.4	0	1200

Table 3.3 Eggs per gram (epg) of faeces in lambs sampled in 2003

Date of sampling	Number of animals	Mean	Std. Error of mean	Minimum	Maximum
August	254	150.7	26.9	0	5325
September	257	271.3	18.3	0	1875
October	253	252.7	14.5	0	1238

The distribution of faecal egg counts in each month in each year was positively skewed. Figure 3.1 (A, B and C) illustrates the skewed distribution of faecal egg counts of *T. circumcincta* among lambs in August, September and October 2001. Most animals had relatively low faecal egg counts, while a few had quite high counts.

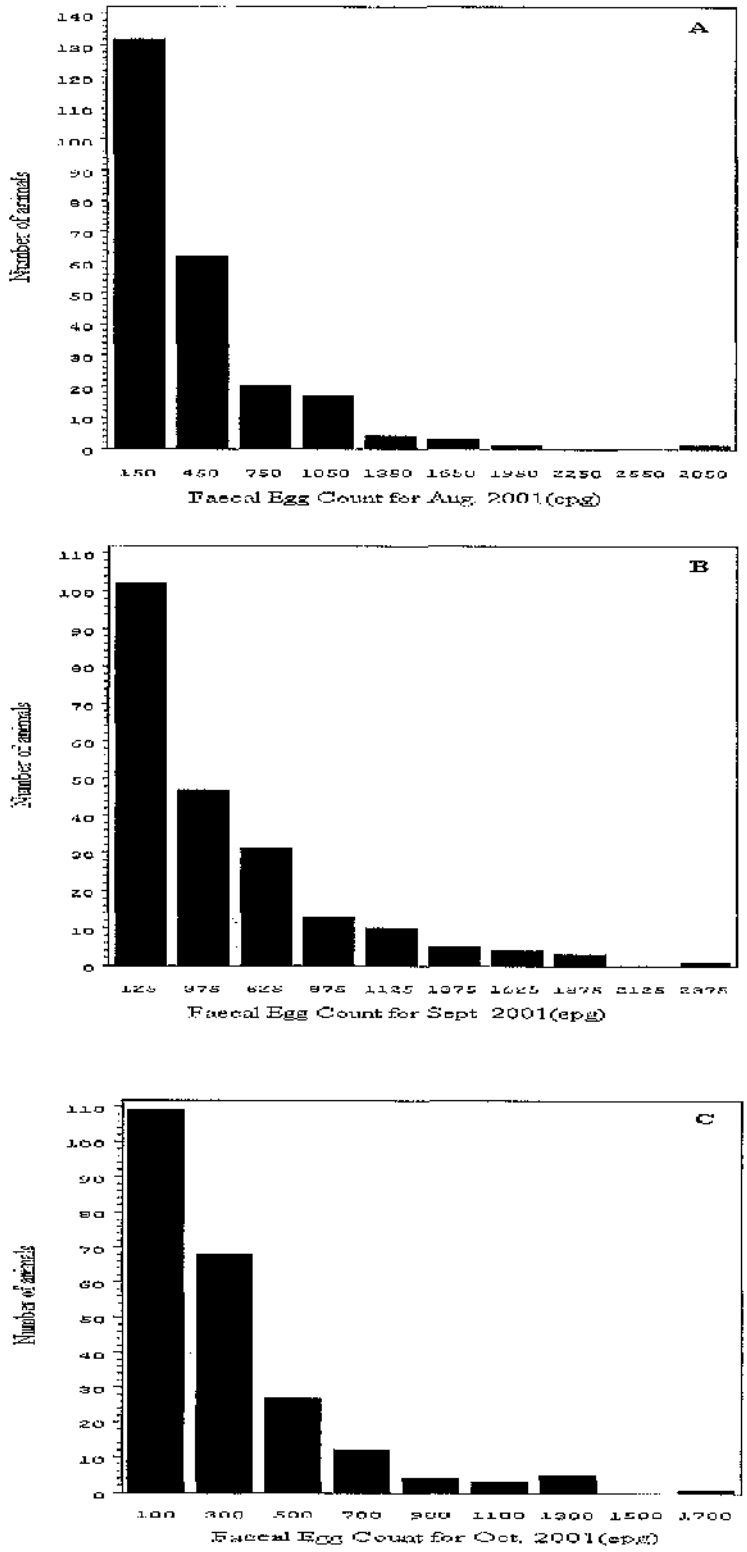


Fig.3.1 Distribution of faecal egg counts in August (A), September (B) and October (C) 2001. In all months the distributions of egg counts were positively skewed indicated that the majority of lambs had relatively low egg counts while a few lambs had quite high counts.

Tables 3.4, 3.5 and 3.6 provide the Pearson correlation coefficients that were calculated from log-transformed faecal egg counts within each month for August, September and October 2001. Pearson correlation coefficient were used to compare each count with the other three counts on the same faecal preparation from the same animal. All correlations were positive and statistically very highly significant ($p< 0.001$), indicating that the repeatability of the technique was high and suggesting that the technique was relatively precise.

Table 3.4 Correlations among transformed faecal nematode egg counts from 4 month old lambs in August 2001.

	Log epg1	Log epg2	Log epg3
Log epg1	-		
Log epg2	0.91	-	
Log epg3	0.93	0.92	-
Log epg4	0.94	0.91	0.93

$P < 0.001$

Table 3.5 Correlations among transformed faecal nematode egg counts from 4 month old lambs in September 2001

	Log epg1	Log epg2	Log epg3
Log epg1	-		
Log epg2	0.90	-	
Log epg3	0.91	0.90	-
Log epg4	0.96	0.95	0.97

$P < 0.001$

Table 3.6 Correlations among transformed faecal nematode egg counts from 4 month old lambs in October 2001

	Log epg1	Log epg2	Log epg3
Log epg1	-		
Log epg2	0.90	-	
Log epg3	0.87	0.90	-
Log epg4	0.93	0.95	0.90

$P < 0.001$

Tables 3.7, 3.8 and 3.9 revealed the correlation among transformed faecal egg counts between month to month in three successive years. Most correlations were positive, but not all were significant. Table 3.7 shows the correlation of transformed faecal egg counts in 2001, there was a highly significant but weak correlation ($r = 0.18$, $p < 0.01$) between August and October, while there was a very highly significant correlation ($r = 0.35$, $P < 0.001$) between August and September, and between September and October ($r = 0.34$, $P < 0.001$).

Table 3.8 shows the correlations of transformed faecal egg counts in 2002. There was a highly significant but very weak correlation between August and September ($r = 0.12$, $P < 0.01$). Counts in September and October showed a very highly significant correlation ($r = 0.25$, $P < 0.001$), while there was no correlation between August and October.

Table 3.9 shows the correlation of transformed faecal egg counts in 2003. There was a very weak non-significant correlation between August and September ($r = 0.07$, $P = 0.24$). A weak but significant correlation ($r = 0.11$, $P < 0.05$), was found among transformed faecal egg counts between September and October. The counts in August and October were weakly but highly significantly correlated ($r = 0.17$, $P < 0.01$).

Table 3.7 Correlations among transformed faecal nematode egg counts from 4 month old lambs in 2001

	August	September	October
August	-		
September	0.35***	-	
October	0.18**	0.34***	-

** $P < 0.01$. *** $P < 0.001$.

Table 3.8 Correlations among transformed faecal nematode egg counts from 4 month old lambs in 2002

	August	September	October
August	-		
September	0.12**	-	
October	0.00	0.25***	-

** $P < 0.01$. *** $P < 0.001$

Table 3.9 Correlations among transformed faecal nematode egg counts from 4 month old lambs in 2003

	August	September	October
August	-		
September	0.07	-	
October	0.17**	0.11*	-

* $P < 0.05$. ** $P < 0.01$.

Parasite burdens have been generally found to be overdispersed, with the negative binomial distribution providing a good empirical description of observed distribution of parasite burdens within host populations. In overdispersed distributions, most hosts carry few parasites, while a few heavily infected hosts harbour a large proportion of the total parasite population.

Tables 3.10, 3.11 and 3.12 present *k* the inverse index of dispersion for the negative binomial distribution and its standard error. The *k* values increased in each year indicating, as k is an inverse index of overdispersion, that overdispersion decreased from August to October through September, in all three years (Table 3.10 and 3.11). Most values of k fell between 0.56 and 1.09 except in August 2003. Table 3.12 shows that, *k* was very low (0.199) at this time. This low value was probably due to the presence of a very high faecal egg count. (5325).

The negative binomial distribution was fitted to the number of faecal eggs counted. In September 2001, faecal egg count samples gave a good fit to the negative binomial, while in August and October samples did not provide a good fit to the negative binomial (Table 3.10). In 2002, the distribution in September was not compatible with the negative binomial, but the other two sets of samples gave acceptable fits (Table 3.11). In 2003, September and October faecal egg samples gave a good fit to the negative binomial, but in August the set did not fit to the negative binomial (Table 3.12). The reason of the poor fit of the negative binomial distribution will be discussed in more detail later (see chapter four).

Table 3.10 Index of dispersion when negative binomial distribution were fitted to faecal egg counts of sheep sampled in 2001

Date of Sampling	Index of dispersion	SE	Goodness-of-fit Probability
August	0.615	0.146	0.07
September	0.668	0.141	0.21
October	0.762	0.126	0.01

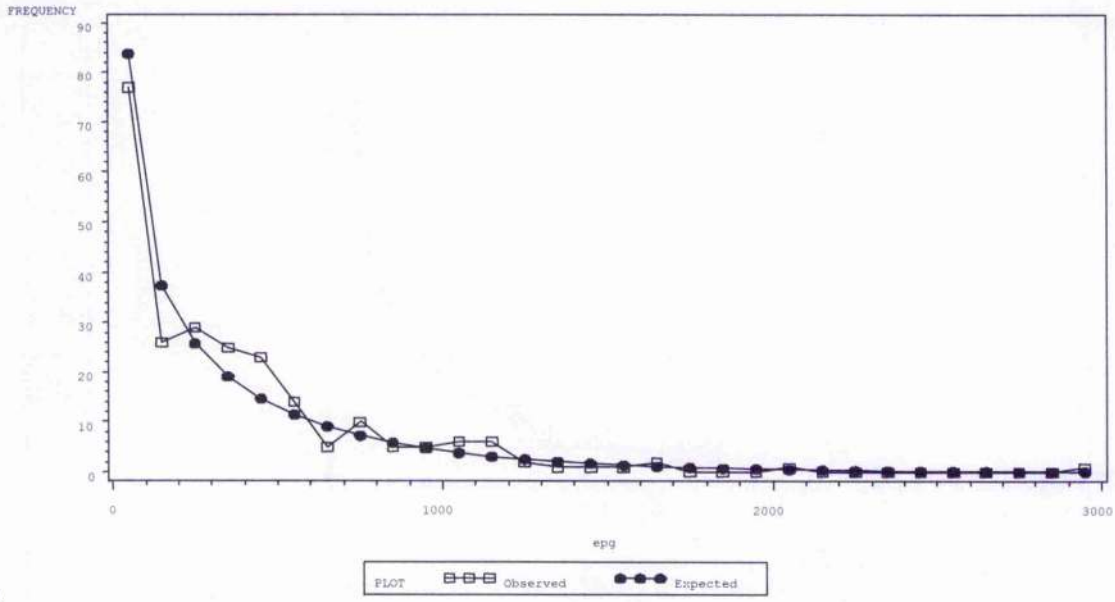
Table 3.11 Index of dispersion when negative binomial distribution were fitted to faecal egg counts of sheep sampled in 2002

Date of Sampling	Index of dispersion	SE	Goodness-of-fit Probability
August	0.561	0.154	0.75
September	0.767	0.116	0.08
October	0.772	0.118	0.28

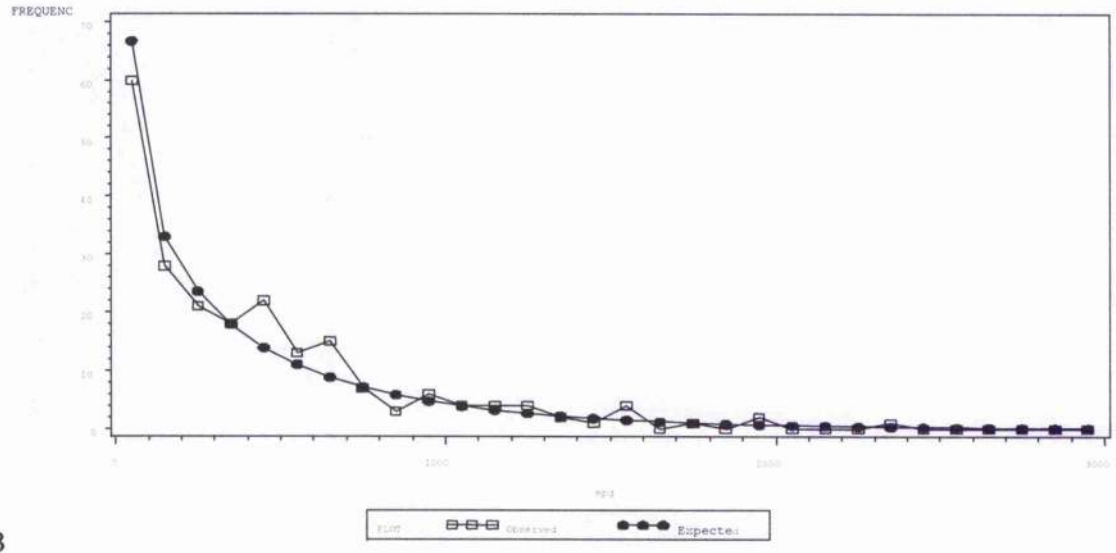
Table 3.12 Index of dispersion when negative binomial distribution were fitted to faecal egg counts of sheep sampled in 2003

Date of Sampling	Index of dispersion	SE	Goodness-of-fit Probability
August	0.199	0.518	0.00
September	0.767	0.117	0.22
October	1.091	0.085	0.16

Fig.3.2 illustrates the negative binomial distribution in all three years. Chi-square tests were used to examine the goodness of fit to the negative binomial distribution

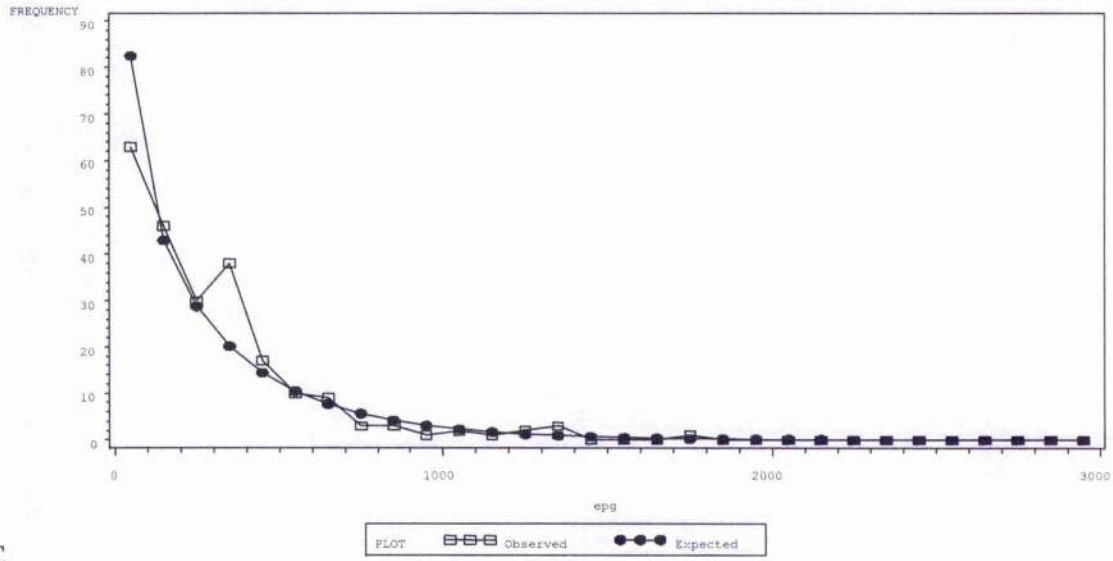


A

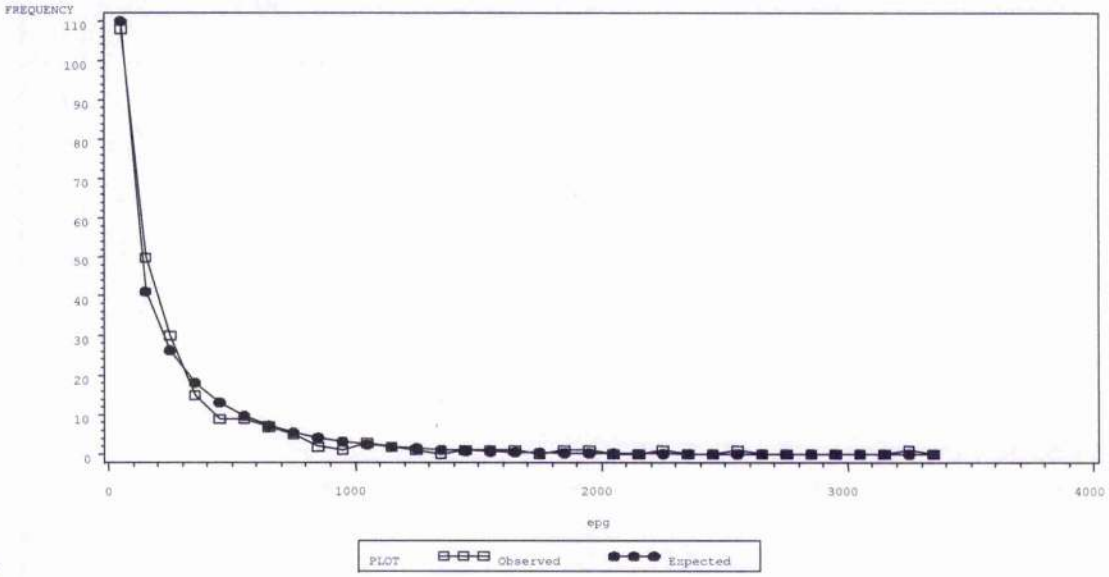


B

Fig 3.2 Negative binomial distribution in (A) August 01 and (B) September 01

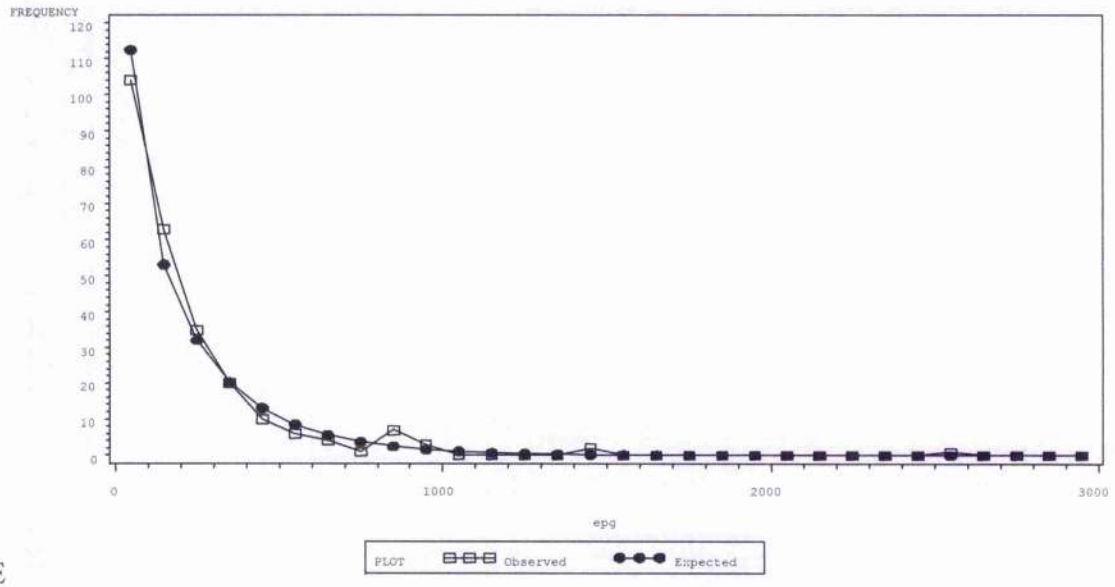


C

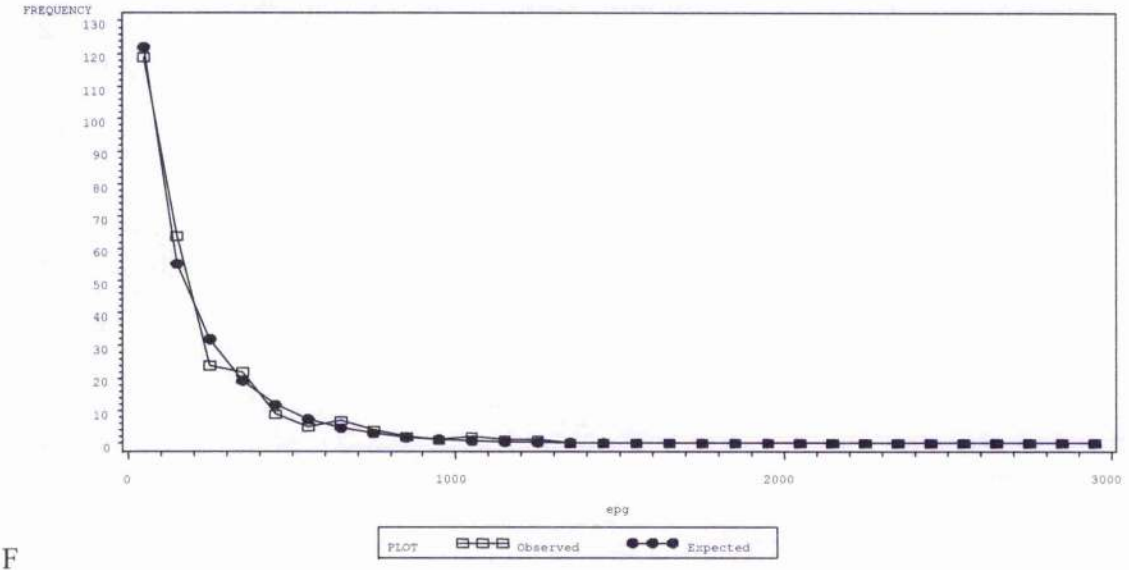


D

Fig 3.2 Negative binomial distribution in (C) October 01 and (D) August 02

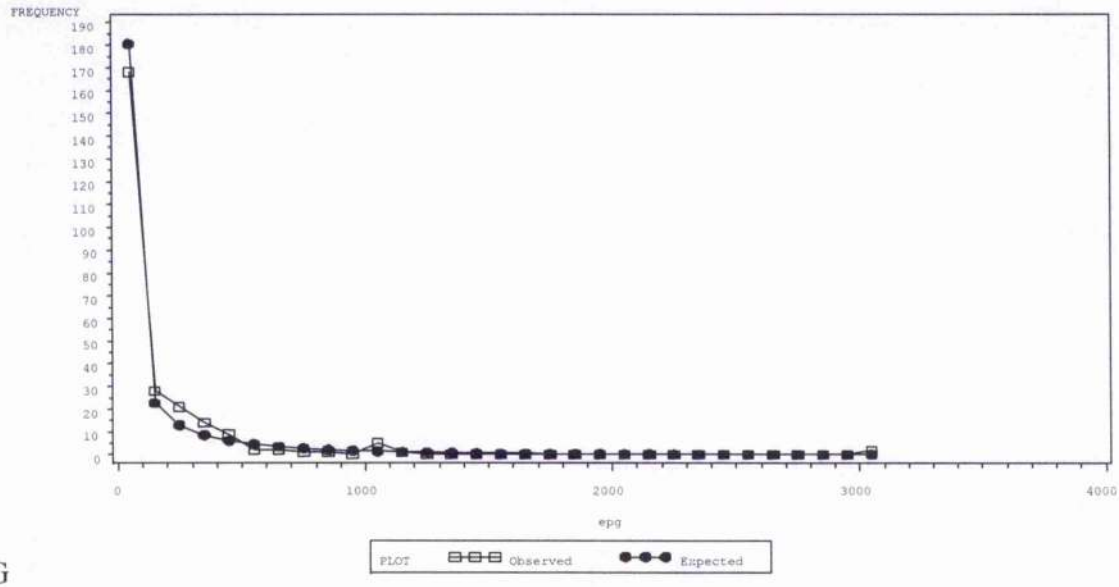


E

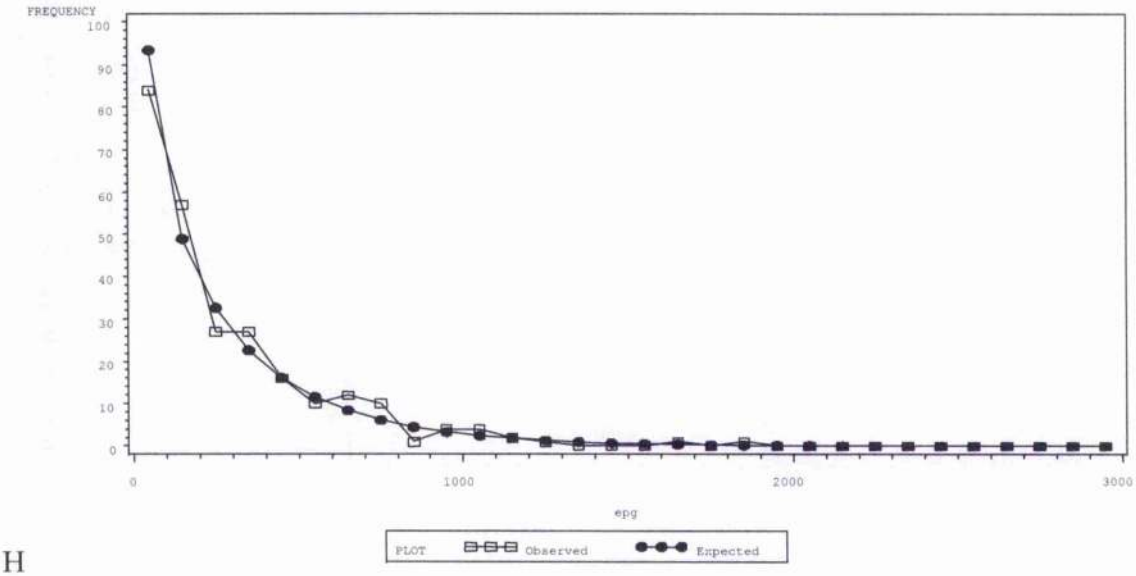


F

Fig 3.2 Negative binomial distribution in (E) September 02 and (F) October 02

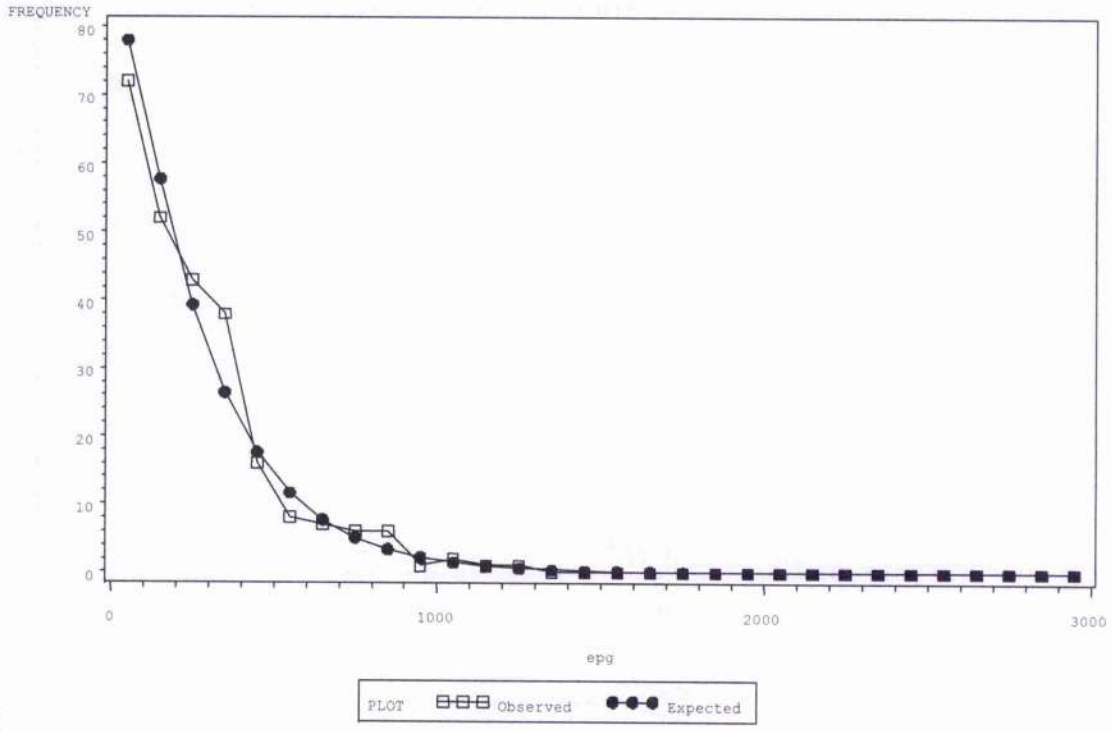


G



H

Fig 3.2 Negative binomial distribution in (G) August 02 and (H) September 02



I

Fig 3.2 Negative binomial distribution in (I) October 03

Pasturel larval counts showed that the overwhelming majority of larvae were *T. circumcincta.* Other larvae such as Nematodirus were observed, but with low counts compared with Teladorsagia (Tables 13, 14 and 15).

Table 3.13 Total pasturel larval counts of third-stage larvae per kilogram-dried herbage (13/kdh) in 2001

		Teladorsagia	Ostertagia	Nematodirus	Cooperia
August	Field 1	12	0	6	0
	Field 2	70	0	14	0
	Field 3	72	0	18	0
September	Field 1	100	130	11	0
	Field 2	56	72	40	0
October	Field 1	143	55	33	0
	Field 2	182	26	52	0

Table 3.14 Total pasturel larval counts of third-stage larvae per kilogram-dried herbage (13/kdh) in 2002

		Teladorsagia	Ostertagia	Nematodirus	Cooperia
August	Field 1	81	40	40	20
September	Field 1	35	0	53	0
October	Field 1	21	0	21	0

Table 3.15 Total pasturel larval counts of third-stage larvae per kilogram-dried herbage (13/kdh) in 2003

		Teladorsagia	Ostertagia	Nematodirus	Cooperia
August	Field 1	55	0	147	36
September	Field 1	164	0	185	65
	Field 2	142	33	44	0
October	Field 1	59	39	78	39

3.4 Discussion

This chapter has investigated the changes in the mean and distribution of faecal egg counts in lambs of Scottish Blackface sheep following natural infection with gastrointestinal nematodes predominantly *T. circumcincta*.

A longitudinal study of faecal egg counts was made over three years at monthly intervals between August to October. This study has shown that there was no discernible pattern to egg counts within each season; each year out of the three had its own pattern. The results on this farm are not strictly comparable with other studies because lambs on this farm were given monthly anthelmintic treatment after each collection of faecal samples. However, the infection pattern approximates those previously described (Boag and Thomas 1973;Thomas and Boag 1972).

The factors that influence these changes in faecal egg counts from month to month and from year to year could include grazing management, anthelmintic treatment and differences in the weather, especially humidity, which influence the number of infective larvae ingested by lambs and the rate of development of eggs to larvae (Stear et al. 1995b).

The mean egg count in October was lower than that in September in each year, which may reflect the development of host resistance (Smith et al. 1985). As such, faecal egg counts may be considered to simply be an indicator of resistance/ susceptibility to parasite infection (Bishop et al. 1996). Therefore using the average of several faecal egg counts in selective breeding, may offer a desirable and feasible option in assisting in the control of nematode infections. However, in other studies the egg count does not always drop from September to October (Stear et al. 2005a), therefore differences in the mean egg counts between September

and October may reflect differences in the number of infective larvae ingested or differences in species composition.

The Pearson product moment correlation coefficients among transformed faecal egg counts between August, September and October for the three years were usually significant but they were relatively small. The correlations were significant between August and September in 2001 and 2002, similar to previous results obtained (Stear et al. 1995b) which indicated that the correlations were significant and positive between August and September and not in the preceding months. The correlations were significant between September and October for all three years. This result means that lambs with high egg counts in September tend to have higher egg counts in October.

The negative binomial is perhaps the most widely used distribution to describe theoretical and empirical parasite burdens (Gregory and Woolhouse 1993). The frequency distributions of faecal egg counts were positively skewed in all nine months from 2001 to 2003, confirming that the most situations, egg excretions derived from natural infection in sheep showed an aggregated distribution and were consistent with a negative binomial pattern. Negative binomial distributions have been fitted to faecal egg counts in sheep (Hunter and Quenouille 1952), (Donald 1968) (Roberts and Swan 1982). Barger (1985) reported that nematode counts for four genera of nematodes also followed negative binomial distributions. However, in the present study, some distributions were not compatible with the negative binomial distribution, this will be discussed further in chapter four.

A comparison of the negative binomial k values (regarded as an inverse measure of the extent of overdispersion) showed that the amount of dispersion decreases as animals become older (for example in August 2001 $k = 0.61$, and in October $k = 0.72$); this result agreed with

(Maizels et al. 1993) who stated the amount of overdispersion is less in the older populations. However, the differences among the k values in our study were not statistically significant and any trends need to be interpreted cautiously. There is a widely cited belief that variation decreases as animal mature because immune responses decrease parasite survival (Maizels et al. 1993). In contrast, this result disagreed with other studies (Stear et al. 1995b) who reported that the amount of overdispersion increases as animal mature.

However, in August 2003 the k the inverse index of dispersion was very low indicating that egg counts were becoming more aggregated within few animals. This low value of k was arises because two of the lambs had very high faecal egg counts (3125 and 5325 epg). These results suggest that a relatively small part of the flocks are responsible for most of the total pasture contamination.

CHAPTER FOUR

Variation in faecal egg counts among populations

4.1 INTRODUCTION

The previous chapter, and other studies, have demonstrated that faecal egg counts are widely used to estimate the relative susceptibility of infected sheep to nematode infection (Bisset et al. 2001;Woolaston and Windon 2001). In cool, temperate climates such as the UK, the dominant nematode is *Teladorsagia circumcincta* (Stear et al. 1998), and faecal egg counts following natural infection are used to guide selection decisions when breeding sheep for nematode resistance. Faecal egg counts following natural infection are very variable both within and between sheep populations (Stear et al. 1995b) but several issues remain unresolved. Faecal egg counts in some sheep populations show a good fit to the negative binomial distribution while others do not (Stear et al. 1995b). However, the reasons for this are unclear. The lack of consistency hinders the application of general linear mixed models for data analysis. The variation among populations has not been quantified yet an assessment of variation would assist the design of selection schemes that use several different farms. Further, some populations show much higher levels of variation among animals than others. Understanding the reasons for this variation would lead to better characterisation of resistant animals and could help to identify the mechanisms underlying resistance.

Therefore this chapter aims to extend the results of the previous chapter and the other studies by investigating the poor fit of the negative binomial to egg counts in lambs, to quantify the variation in faecal egg counts among populations and to examine the sources of this variation.

4.2 MATERIALS AND METHODS

4.2.1 Animals

The sheep came from two upland farms in Scotland; one in west central Scotland and one in the Borders region in southeast Scotland. The sheep were all straightbred Scottish Blackface. The lambs were born in late spring (April and May) and weaned at 3 or 4 months of age. The

management regime on the first farm has been described previously (Stear et al. 1995b). On this farm, the lambs were sampled (faecal and blood samples) every 28 days from 8 to 24 weeks of age from 1990 to 1996. Additionally, lambs born in 1992 to 1995 were also sampled at 4 weeks of age. All the animals on the first farm were necropsied at 30 or 31 weeks after the last sample date. All lambs were given a broad spectrum anthelmintic (albendazole sulphoxide) according to the manufacturer's recommendations every 4 weeks from 4 to 20 weeks of age. The animals from the second farm are described in chapter three (see section 3.2.1). Each of the seven cohorts from the first farm consisted of 200 lambs while each of the three cohorts from the second farm had approximately 250 lambs. Only 70-95% of lambs were sampled on most occasions due to deaths, missing records, lost tags and insufficient faeces in the sample. As the four populations sampled at necropsy contained fewer lambs than the other populations they were not included in the initial assessment of means and variances.

4.2.2 Parasitological methods

The parasitological method is described in chapter two. Briefly faecal egg counts were made according to a modifyied McMaster method with saturated salt solution. Each sample was counted on four separate McMaster chambers and each egg counted represented 50 eggs per gram. These counts will be referred to as epg1, epg2, epg3 and epg4. The dominant nematode on this farm was *T. circumcincta.* In addition replicate aliquots (to improve the precision of the technique) from the same faecal preparation were counted in September 1993, October 1993, October 1994, and all samples from 1995 onwards. Standard parasitological procedures were used at necropsy to identify and count all nematodes present in the abomasum and small intestine (Armour et al. 1966;Stear et al. 1998) in lambs from the first farm. The large intestine was not examined as the frequency of anthelmintic treatment would prevent any large intestinal parasites surviving to the egg laying stage (Stear et al. 1998).

4.2.3 Statistical analysis

The SAS suite of statistical programs version 9.1 was used for all analyses (SAS Institute, Cary, N. Carolina). The univariate procedure was used to estimate means, variances, standard deviations and ranges for each population sampled on each occasion. When replicate aliquots were counted only the first aliquot was used to estimate means, variances and the mean-variance relationship. There was an outlying population with a relatively low egg count of 201 epg but a high variance of 831,550 epg^2. This sample was taken in May 1994 and comprised only 88 lambs. All other samples had over 140 animals. This outlying population was discarded from further analyses. The distributions of means and variances were both skewed to the right. Gamma distributions were fitted to the data with the Capability procedure in the SAS/QC suite of programs. The gamma and lognormal distributions have three parameters: threshold, scale and shape. The threshold parameter was set to zero while maximum likelihood estimates of the scale and shape parameters were calculated iteratively by the Newton-Raphson approximation. Goodness of fit was tested by the Anderson-Darling statistic; this test belongs to the quadratic class of empirical distribution function statistics (D'Agostino and Stephens 1986).

The relationship between the mean and variance was estimated by fitting a regression line between log transformed variance and log-transformed mean: log(variance) = $\alpha + \beta$ *log(mean) (Perry 1981;Taylor 1961). This regression was fitted with the GLM procedure in SAS. When back-transformed, this gives a power relationship of the form: variance = a * mean **b.

Among nematode eggs, only Nematodirus spp. are counted separately. Eggs from the other species cannot be distinguished from each other and the results on their eggs are poled. Multiple regression with the SAS GLM program was used to examine the relationship

between the non-Nematodirus egg count and the number of nematodes present from the five most common taxa: *Teladorsagia circumcincta*, Cooperia spp., *Trichostrongylus vitrinus*, *Trichostrongylus axei* and *Haemonchus contortus*.

There is a nonlinear relationship between the number of adult *T. circumcincta* in the abomasum and the number of eggs produced by this species (Bishop and Stear 2000b;Stear and Bishop 1999). The egg output depends upon the number of adult nematodes and their mean egg output. The mean egg output is strongly associated with the mean length of the adult female worms (Stear and Bishop 1999). The mean egg output per worm was estimated as worm length to the power 0.4 multiplied by 1.12; one was subtracted from the sum. The predicted egg output was then calculated by multiplying the total number of adult worms by the mean egg output per worm. The predicted egg count for *T. circumcincta* was subtracted from the observed egg count to create a residual egg count. Multiple regression was then used to examine the relationship between the residual egg count and the number of nematodes from the four taxa: *Cooperia* spp., *T. vitrinus*, *T. axei* and H. *contortus*. Due to the presence of negative numbers 1000 was added to all residual counts prior to log transformation.

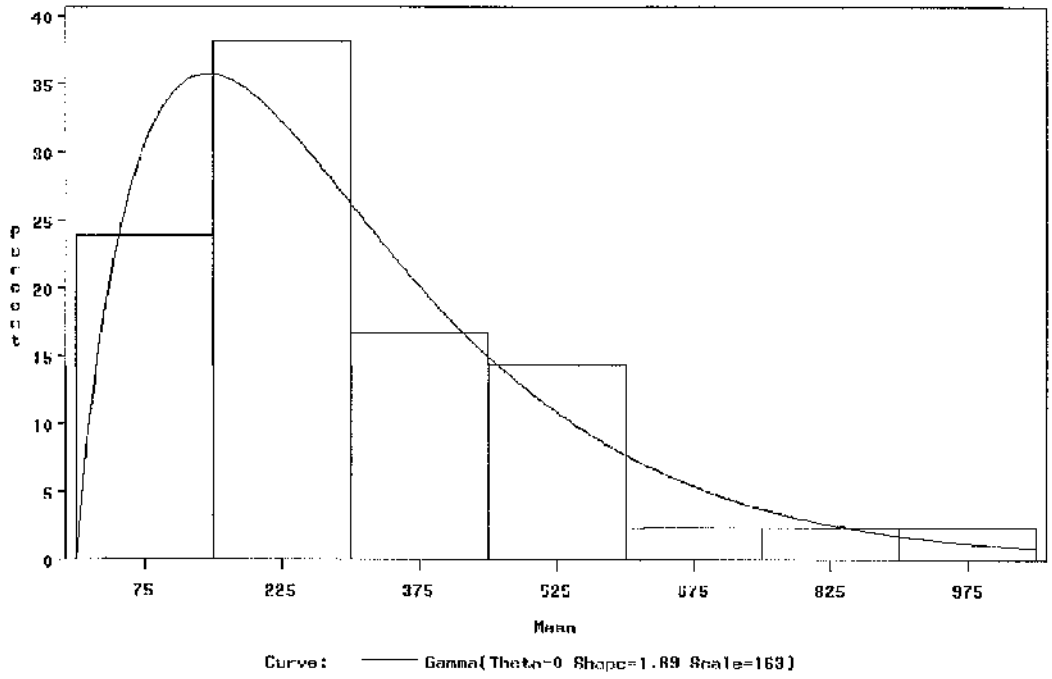
Generalised linear modelling was carried out with the GLIMMIX macro in SAS (Littell et al. 1996).

4.3 RESULTS

Fig 4.1 shows the distribution of mean egg count among the populations sampled from the two farms. Each of the 42 data points is the mean egg count for a particular cohort on one farm at a single date. The distribution was right skewed with a mean of 305 and a median of 255 eggs per gram. The data appeared to follow a gamma distribution (Anderson-Darling statistic $p > 0.50$). Maximum likelihood estimates for the scale and shape parameters were

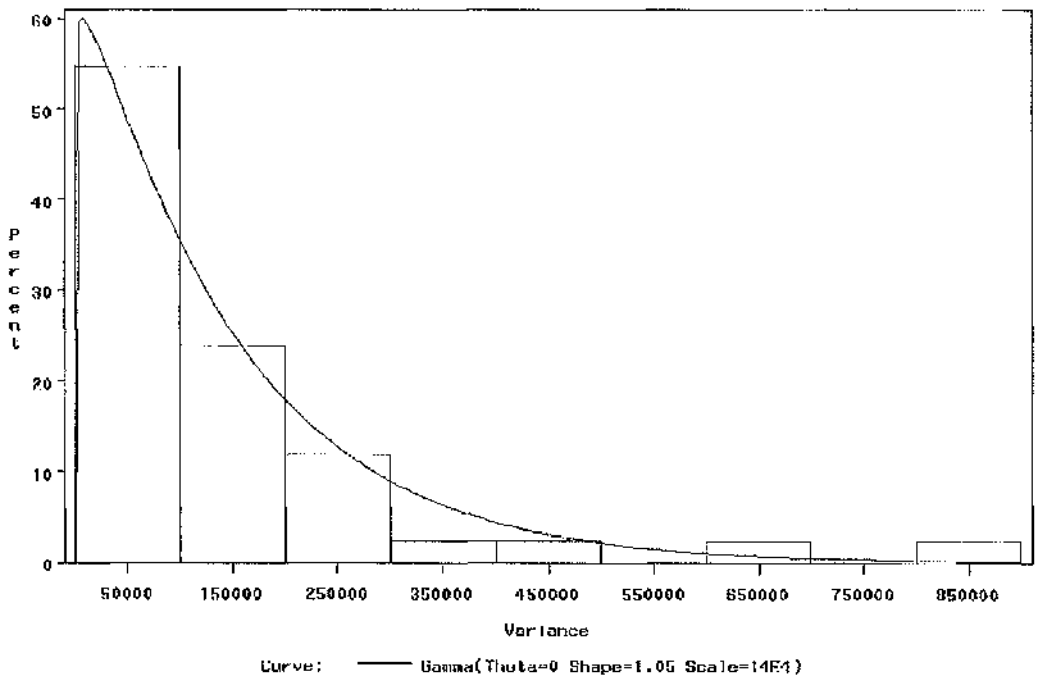
163 and 1.89, respectively. The gamma distribution with these parameters has been superimposed on the histogram (Fig 4.1).

Fig 4.1. The gamma distribution of mean egg counts among the populations



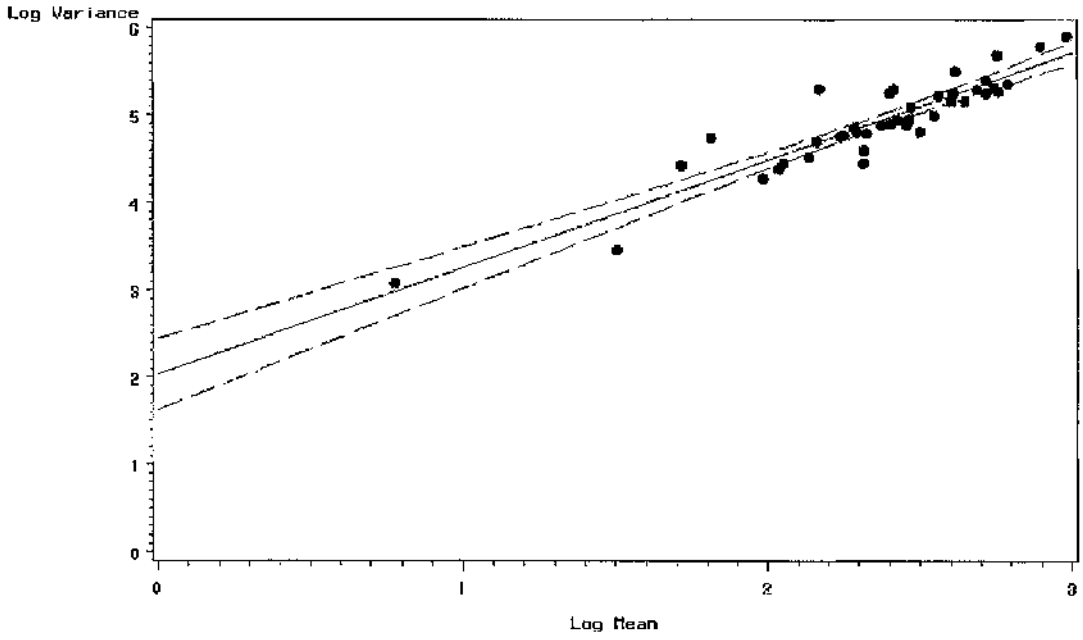
The distribution of the variances of the egg counts is presented in Fig 4.2. This distribution was also right skewed. Most populations had relatively small variances but a small number had quite high variances. The median variance was 89890 and the mean variance was higher at 162743 eggs per gram^2. As with the distribution of means, the distribution of variances among the sampled populations appeared to follow a gamma distribution (Anderson-Darling statistic p = 0.169). Maximum likelihood estimates for the scale and shape parameters were 139462 and 1.05. The gamma distribution with these parameters has been superimposed on the histogram (Fig 4.2).

Fig 4.2 The distribution of egg count variances among the populations



Regression analysis (Fig 4.3) demonstrated that the variance = 120 * (mean epg) $^{1.23 \pm 0.08}$. The 95% confidence limits on the scalar term were 39 and 372. Fig 4.3 illustrates this relationship between the mean and the variance. The R-square value was 0.84, indicating that variation among populations (between the two farms) in their variances largely reflected variation among populations in their mean egg counts.

Fig 4.3 The relationship between transformed egg count mean and variance. Means and variances were transformed by taking logarithms to the base 10. The solid line represents the regression and the dotted lines represent 95% confidence limits.



An additional four populations from farm one were examined at necropsy. Table 4.1 presents the mean egg count and variance for these four populations as well as the number of species present. As the numbers of fourth and fifth-stage larvae do not influence the egg count they have not been included in table 4.1.

Table 4.1 The mean and variance of faecal egg count and the mean number of adult nematodes present at necropsy.

Year	Number of Lambs	Mean Epg	Variance Epg^2	*T. circumcincta*	*T. axei*	*H. contortus*	Cooperia *spp.*	*T. vitrinus*	*B. trigonocephalum*
1992	110	87	22,359	6538	0	0	74	246	0.9
1993	100	317	65,306	2778	65	0	350	114	0
1994	169	494	218,000	1554	1.2	0	523	246	0
1995	151	1767	2,710,068	3000	101	5	4382	1020	0

Larvae were found for only *T. circumcincta*, *Cooperia* spp. and *T. vitrinus*. Most fourth-stage larvae will be inhibited but a small number may arise from recent infection. In 1992, the mean numbers of fourth-stage and fifth-stage larvae in each lamb were respectively 5738 and 574 for *T. circumcincta*, 2 and 7 for *Cooperia* spp. and 4 and 5 for *T. vitrinus*. In 1993, mean numbers of fourth-stage and fifth-stage larvae were respectively 528 and 100 for *T. circumcincta*, 18 and 19 for *Cooperia* spp. and 1 and 1 for *T. vitrinus*. In 1994 and 1995 all recovered larvae were *T. circumcincta*. There were 705 and 31 fourth and fifth-stage larvae in 1994 and 3221 and 82 respectively in 1995.

Table 4.1 demonstrates that the variance increased as the egg count increased, in line with the previous analysis. The interesting feature of table 4.1 is that high egg counts and high variances are not due to high numbers of adult nematodes *per se* but to high numbers of species other than *T. circumcincta*. For example, the lowest egg counts (87 epg) occurred in 1992. This year had the second highest total of nematodes (6860) but over 95% of these were *T. circumcincta*. In contrast, the mean egg counts were much higher in 1993 (317 epg) and 1994 (494 epg) but the number of adult nematodes was much lower at 3309 and 2336 respectively. However, the proportion of *T. circumcincta* was lower at 84% in 1993 and 66% in 1994. Together, these results suggest that high means and variances in faecal egg counts in October at the end of the grazing season are not due to number of nematodes but to the presence of species other than *T. circumcincta*.

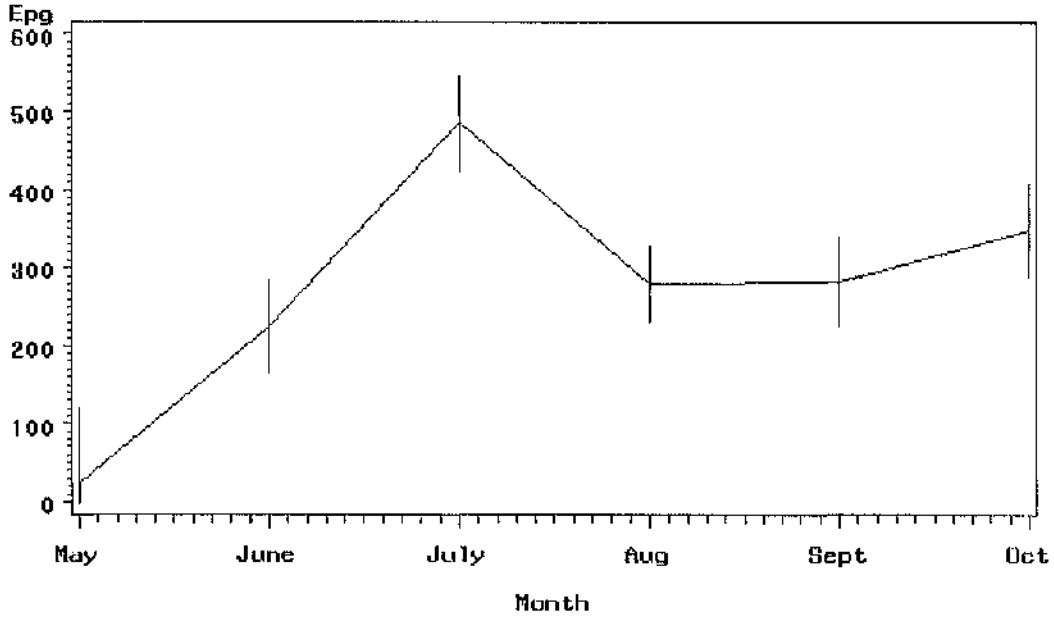
Multiple regression was used to examine the relationship in these lambs in October between the total faecal egg count and the number of adult parasites of the five taxa (*T. circumcincta*, *Cooperia* spp., *T. vitrinus*, *T. axei* and *H. contortus).* Both faecal egg count and adult parasite numbers for each species were transformed by log10(x+1). The initial analysis showed a negative relationship (-0.23 $\pm$ 0.09; $p < 0.05$) between faecal egg count and transformed number of *T. circumcincta* and positive relationships between transformed egg count and transformed number of *Cooperia* spp. (+0.33 $\pm$ 0.03; $p < 0.001$) and the transformed number of *T. axei* (+0.15 $\pm$ 0.07; $p < 0.05$). The relationships between faecal egg count and the transformed numbers of *T. vitrinus* and *H. contortus* were not significant (p=0.21 and p=0.50) respectively.

The *T. circumcincta* egg count was predicted from the number of adult *T. circumcincta* using previous published methods (Stear et al. 1998). This predicted egg count was then subtracted from the actual egg count and the residual egg count transformed by log 10(residual+1000). Multiple regression analysis demonstrated highly significant effects between transformed residual egg count and the transformed numbers of *Cooperia* spp. (+0.07 $\pm$ 0.01; $p < 0.001$), *T. axei* (+0.04 $\pm$ 0.01; $p < 0.05$) and *T. vitrinus* (+0.03 $\pm$ 0.01; $p < 0.001$). The relationship between faecal egg count and the transformed number of *H. contortus* was not significant (p=0.64), possibly because only 8 of 483 lambs examined were infected with this parasite.

Generalized linear modelling with a gamma distribution and a reciprocal link function was used to test the relationship between mean egg count and both farms, year and month. Three separate univariate analyses were carried out and each variable was fitted separately as a fixed effect. These analyses showed that there were no significant differences in mean egg count between the two farms (p=0.455), a nonsignificant and inconclusive result for year (p=0.053) and significant differences among months (p=0.024). Egg counts were low in May, rose in

June, peaked in July, fell in August, remained stable in September but rose again in October (Fig 4.4). The highest July mean egg counts occurred in 1993 (572 epg).

Fig 4.4 Egg count means plus standard errors by month of sampling. Lambs were born in a 3-week interval then sampled every 28 days from 1992 to 1995. All lambs were treated with anthelmintic at each sample date.



4.4 DISCUSSION

There was considerable variation among the populations sampled in faecal egg count means and variances. The distribution of means and variances were both skewed. Most populations had relatively low means and variances but a small proportion had high means and variances. The variance was related to the mean to the power 1.23; this exponent was significantly greater than 1.0 and significantly less than 2.0. Analysis of necropsy data suggested that high means and variances were not simply due to high intensities of infection but to the presence of species other than *T. circumcincta,* particularly *Cooperia* spp. and *Trichostrongylus vitrinus.*

The contribution of other nematode species to high egg counts is consistent with previously published reports on the density-dependent regulation of fecundity in *T. circumcincta* (Bishop and Stear 2000b). As the intensity of infection with *T. circumcincta* increases, an increasing number of larvae arrest development while those that do develop into adults produce fewer eggs per day. Previously published results hav been used to predict egg output from the number of adult parasites (Bishop and Stear 2000b;Stear and Bishop 1999). After subtracting this predicted output from the observed egg count, multiple regression analysis on the transformed residual egg counts gave highly significant positive relationships with the numbers of *Cooperia* spp., *T. axei* and *T. vitrinus.* Care is needed in interpreting these results because the residual egg count is an imprecise estimate of the egg count due to species other than *T. circumcincta.* Nonetheless the conclusion that the egg count is influenced by all nematodes present is plausible. Although *T. circumcincta* is the predominant species, the egg count does not necessarily reflect this. Indeed lambs with many adult *T. circumcincta* produce fewer nematode eggs than lambs with moderate infections (Bishop and Stear 2000b)

There was no significant difference in mean egg counts between the two farms sampled. However, the mean egg counts varied with the month of sampling. Egg counts rose to a peak

in July then declined before rising again in October. A similar bimodal pattern with slightly earlier timings was reported previously for untreated lambs (Thomas and Boag 1972). This study was carried out under commercial conditions with regular anthelmintic usage; therefore the values observed each month represent independent infections and are not influenced by pre-existing infections derived from previous months.

The decline from the first peak has been explained by the onset of immunity in lambs (Stear et al. 1999b). However, part of the peak could be contributed by time-dependent variation in other nematodes such as *Cooperia* spp. Necropsies of large numbers of infected lambs at regular intervals during the grazing season are needed to clarify the contribution made by different species of nematodes.

Faecal egg counts in sheep are not particularly well-described by the negative binomial distribution (Stear et al. 1995b) this lack of fit is surprising because the negative binomial distribution is a flexible distribution that is widely used to describe parasite distributions among hosts (Bliss and Fisher 1953;Hunter and Quenouille 1952). The poor fit of the negative binomial distribution may be explained by the observation that several nematode species contribute to the egg count. The dominant nematode is *T. circumcincta* but other species can contribute to the egg count. If each species egg counts follow a negative binomial distribution, the combined distribution would not conform to a negative binomial (Grafen and Woolhouse 1993). In addition, males have higher egg counts than females and this too could lead to departures from the negative binomial distribution (Stear et al. 1995b).

There was a strong relationship between the mean and the variance for egg counts. This relationship followed Taylor's power law (Taylor 1961). Taylor's power law has been used previously in a subset of these data (Stear et al. 1998). Then the regression line was drawn

through the origin but visual examination (Fig 4.3) of the larger data set analysed here suggests that an intercept was more appropriate. Estimating the slope of the regression line is subject to error (Boag et al. 1992;Perry 1981) because both the mean and variances are estimates of the true values. However, there is no agreement on the best way to avoid this problem (Sokal and Rohlf 1995). A coefficient of 1 is consistent with a Poisson distribution and implies a square root transformation while a coefficient of 2 implies a logarithmic transformation is most appropriate. Here the estimate lies between 1 and 2, implying that neither transformation is ideal.

Taylor's Power law has been widely used to describe the relationship between variability in population size and mean abundance of a species over space and time (Anderson et al. 1982;Keeling 2000). Taylor (1961) considered the scalar to be of less importance than the exponent, which generally lies between 1 and 2. Mathematical modelling suggested that the value of the exponent was determined by relative magnitude of birth, death, immigration and emigration rates (Anderson et al. 1982)while others argued that negative interactions among species interactions could produce exponents between one and two (Kilpatrick and Ives 2003).

In conclusion, faecal egg counts vary in naturally infected sheep and mean egg counts vary among different populations and among the same population sampled at different times. The variance was largely determined by the mean and high means are not necessarily due to high intensities of infection but probably reflect the contribution of species other than *T. circumcincta*.

CHAPTER FIVE

THE RELATIONSHIPS BETWEEN IgA ACTIVITY AGAINST THIRD-STAGE LARVAE OF *T. CIRCUMCINCTA*, FAECAL EGG COUNTS AND GROWTH RATE OF SCOTTISH BLACKFACE LAMBS

5.1 Introduction

Breeding for resistance to infection is one of the major foci of research in veterinary parasitology. Due to the emergence of anthelmintic resistance in parasite populations, non-chemotherapeutic methods are being investigated to control *T. circumcincta* infection (Beh and Maddox 1996). A detailed understanding of the immune mechanisms involved in resistance to infection will lead to more sustainable methods of control and in particular will aid in the identification of resistant animals.

Young lambs appear to be unable to control worm burdens (Stear et al. 1996b). Sheep regulate worm length before they regulate worm numbers (Seaton et al. 1989). The only mechanism to have been consistently associated with reduced worm length is the local IgA response (Smith et al. 1985;Stear et al. 1995c). The strength of the association between IgA and parasite fecundity led to hypothesis that the specificity and activity of local IgA was the major mechanism regulating the fecundity of *T. circumcincta* (Stear et al. 1996b) and a major mechanism of resistance to infection in lambs (McCririe et al. 1997;Stear et al. 1995b;Stear et al. 1997;Strain and Stear 1999). The most important manifestation of immunity in growing lambs is the control of worm growth, which causes a reduction in worm fecundity (Stear et al. 1997).

The response to selection could be improved by the use of additional markers. There are a wide variety of potential markers and perhaps the most useful is the IgA response to fourth-stage larvae. Following both deliberate and natural infection, individuals with increased amounts of IgA specific for fourth-stage larvae have shorter worms and lower egg counts (Stear et al. 1995c). This association appears so strong and consistent that IgA may be more useful than faecal egg counts as a marker of susceptibility to infection. In addition, there is an

association in cattle between increased abomasal IgA responses and reduced egg production by *O. ostertagi* (Claerebout and Vercruysse 2000).

Antibody against L_3 antigens tends to reflect both the level of challenge and the innate ability of the individual to respond whereas, antibodies against L_4 and adult stages of the nematode *T. colubriformis* tend to reflect the presence of the nematode in the gut (Douch et al. 1996)

There is a close correlation between the degree of retardation of the parasites and the total IgA response (Smith et al. 1985). They suggested that IgA can block enzymes essential for the normal feeding mechanisms of the larvae. This adds to the suggestion that IgA acts on parasite growth through reducing its ability to feed. Results from several New Zealand trials have indicated that antibody levels (particularly IgG1) to excretory/secretory antigens of L_3 nematodes such as *Trichostrongylus colubriformis* may be a phenotypic marker. Levels of antibody against L_3 antigens are also independent of on-farm drenching strategies (Douch et al. 1996).

A comparison of IgA responses to third-stage, fourth-stage and adult *T. circumcincta* indicated the strongest association with reduced worm length, with increased responses to fourth-stage larvae (Stear et al. 1995c). The responses to third-stage larvae were correlated with the responses to fourth-stage larvae (Sinski et al. 1995a). IgA activity against fourth stage larvae probably is the best phenotypic marker, but recovering fourth-stage larvae requires killing sheep. Third-stage larvae can be recovered from faecal cultures, making responses to third-stage larvae cheaper and easier to measure and killing animals can be avoided. There appear to be very few studies that have examined IgA activity against third-stage larvae as a phenotypic marker for resistance in naturally infected sheep.

The present investigation was undertaken to determine the relationships between IgA activities against third-stage larvae of *T. circumcincta*, faecal egg counts and growth rate of Scottish Blackface lambs and to explore whether plasma IgA activity against third stage larvae could be used as indicator trait for resistance or susceptibility in sheep.

5.2 Materials and Methods

5.2.1 Animals

Seven hundred and fifty nine, naturally-infected six-month old Scottish Blackface lambs were sampled for blood and faeces in October 2001, 2002 and 2003.

5.2.2 Parasitological methods

The parasitological methods are described in Chapter two

5.2.3 ELISA assays

Parasite specific host plasma IgA activities to infection were measured by simple indirect ELISA as described in Chapter two.

5.2.4 Statistical analysis

The Univariate program in the SAS package (SAS Institute, Cary, NC, USA) was used to estimate the means and variances of faecal egg counts in October 2001 to 2003. The association between IgA activity, faecal egg counts and growth rate was estimated by correlation coefficient using the correlation procedure in the SAS statistical package. Egg counts were transformed prior to statistical analysis by taking the logarithm of the egg count plus 10.

5.3 Results

IgA activity against an extract of third-stage larvae was measured using an indirect ELISA and expressed as a percentage of a standard value. Fig. 5.1 shows the regression between replicate measurements of IgA activity in October 2001. The two measurements were carried out to evaluate the repeatability between ELISAs with the same samples on different dates. The regression shows that the two measurements gave similar results. The correlation between the first and the second run was positive and very highly significant ($r = 0.80$, $p < 0.001$).

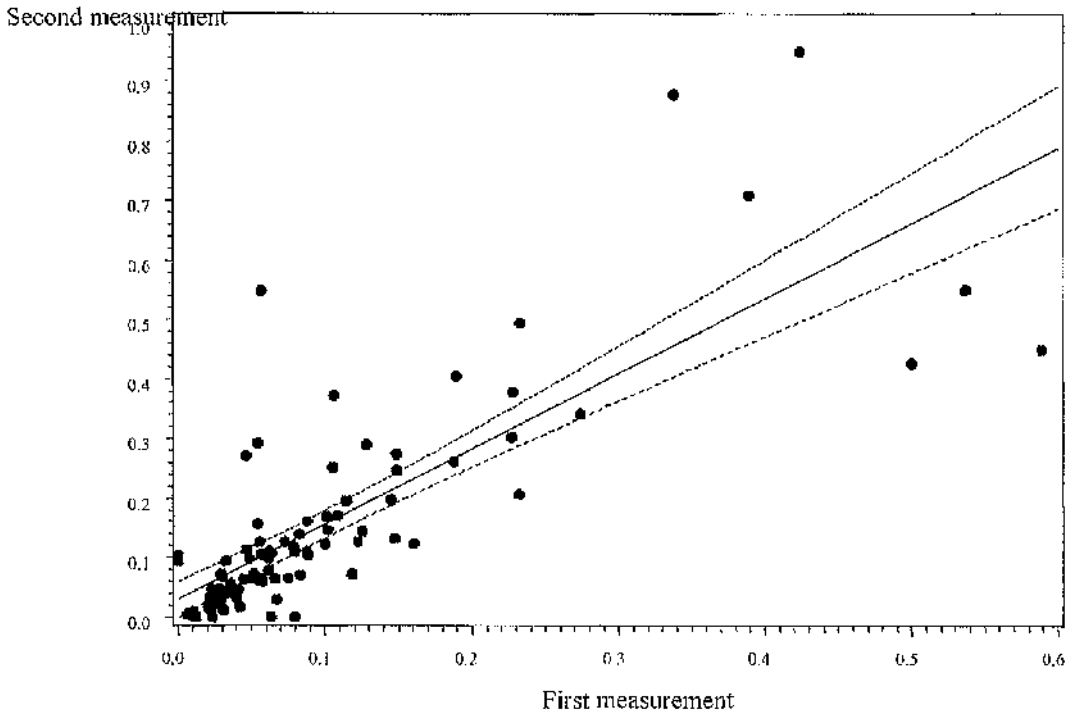
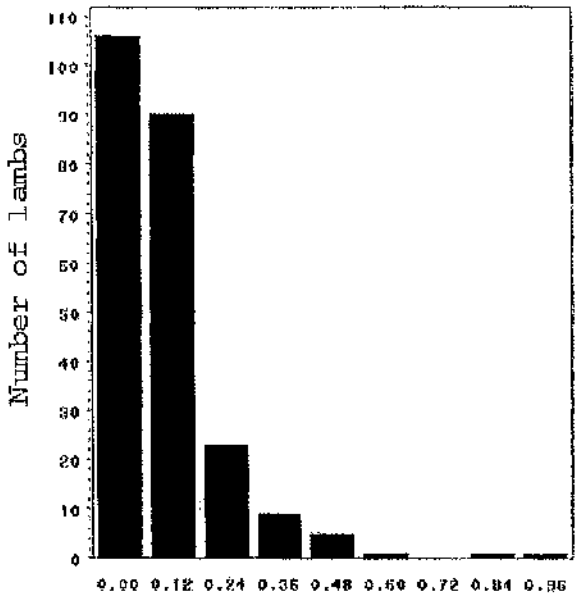


Fig. 5.1 Regression between replicate measurements of IgA activity against third-stage larvae.

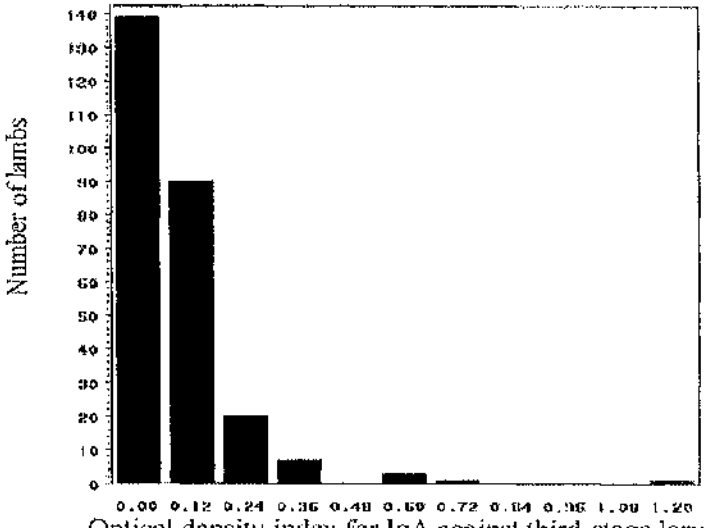
The distribution of IgA activities against third-stage larvae was positively skewed in each year; most lambs had relatively low values but some lambs had quite high values. Fig.5.2 shows the distribution of IgA activity against L_3 of *T. circumcincta* as measured by simple indirect ELISA in the lambs sampled in October 2001, 2002 and 2003.

A



Optical density index for IgA activity against third-stage larvae in 2001

B



Optical density index for IgA against third-stage larvae in 2002

C

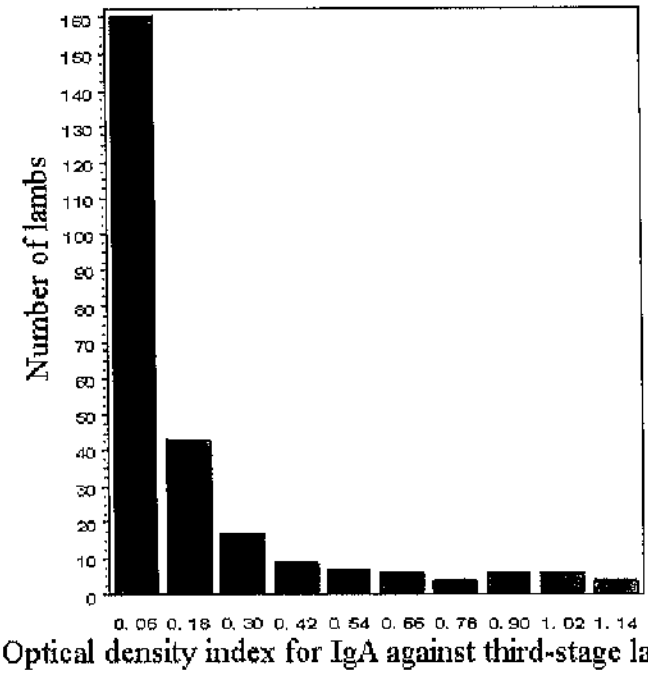


Fig. 5.2 Distribution of plasma IgA activity against third stage larvae of *T. circumcincta* measured by simple indirect ELISA in naturally infected lambs during 2001(A), 2002(B) and 2003(C)

Table 5.1 shows the number of animals, mean IgA activity, standard error of mean, minimum and maximum value observed in October 2001, 2002 and 2003. The mean plasma IgA activity was 0.11 in October 2001, 0.09 in October 2002 and 0.19 in October 2003. Mean IgA activity varied among years but the standard errors are quite small. The mean IgA optical density indices ranged from 0-1.02 in 2001, 0-1.24 in 2002 and 0-1.12 in 2003.

Table 5.1 Mean and standard error of IgA activity against third-stage larvae of *T. circumcincta* in 2001, 2002 and 2003

Date of sampling	Number of animals	Mean of IgA activity	Std. Error of mean	Minimum	Maximum
October 2001	236	0.108	0.008	0.000	1.02
October 2002	261	0.091	0.007	0.000	1.24
October 2003	262	0.186	0.000	0.000	1.12

Table 5.2 shows the mean faecal egg count in October 2001, 2002 and 2003 was 278.2, 181.6 and 252.7 respectively.

Table. 5.2 Eggs per gram of faeces in lambs sampled in 2001, 2002 and 2003

Date of sampling	Mean	Std. Error of Mean	Minimum	Maximum
October 2001	278.2	19.1	0	1700
October 2002	181.6	13.4	0	1200
October 2003	252.7	14.5	0	1238

Table 5.3 provide the Pearson correlation coefficients that were calculated from log-transformed faecal egg counts within each year in October 2001 ($r = 0.014$, $p = 0.826$), 2002 ($r = -0.091$, $p = 0.147$) and 2003 ($r = 0.067$, $p = 0.286$) against the log. transformed optical density indices of IgA activity. The results confirm that there was no significant correlation between faecal egg counts and IgA activity against third-stage larvae.

Table 5.3 Correlations between transformed faecal egg counts and IgA activity in 2001, 2002 and 2003

	Log epg Oct 2001	Log epg Oct 2002	Log epg Oct 2003
IgA activity	0.014	-0.091	0.067

Table 5.4 shows that there was no significant correlation between IgA activity and growth rate at 24 weeks old lambs for the years 2001 ($r = -0.079$, $p = 0.248$), 2002 ($r = -0.143$, $p = 0.021$) and 2003 ($r = -0.042$, $p = 0.503$)

Table 5.4 Correlations between growth rate at 24 weeks old lambs and IgA activity in 2001, 2002 and 2003

	weight in 2001	weight in 2002	weight in 2003
IgA activity	-0.079	-0.143	-0.042

5.4 Discussion

Plasma IgA activity was measured against third-stage larvae of *T. circumcincta* in naturally infected sheep, using a simple, indirect ELISA. The results show that the distribution of plasma IgA activity against third-stage larvae in all lambs was positively skewed with the majority of lambs having relatively low activity but a minority lambs had quite high activity (Fig 5.2). The distribution is similar to the distribution of IgA activity against fourth-stage larvae of *T. circumcincta* (Strain et al. 2002). Serum IgA is dimeric in ruminants and in experimental studies almost totally derived from the gastrointestinal tract (Sheldrake et al. 1984). These results suggest that plasma IgA activity against third-stage larvae may provide a window on local IgA responses in sheep.

No attempt has been made to quantify the amount of IgA in plasma samples. The optical density depends upon the amount of IgA present and the avidity and affinity of IgA for the component of the antigen preparation. As the antigen preparations were complex mixtures, any attempt to estimate absolute antibody concentrations would have been tedious and prone to error (Sinski et al. 1995). The mean optical density indices of plasma IgA activity did not show large differences among the values in the three years.

IgA activity in the serum was dependent upon IgA activity in the abomasum and also the number of adult nematodes present in the abomasums (Sinski et al. 1995a). In addition, there was a strong positive relationship between responses to third-stage and to fourth-stage larvae. The correlation coefficients ranged from 0.60 to 0.79 ($P < 0.001$) for parasite-specific IgA, which showed that sheep with strong responses to third-stage larvae tended to have stronger responses to fourth-stage larvae. Correlated results for the same animals could be due to the existence of some shared, or similar, antigens in the different larval stages. Correlations between responses do not necessarily imply similar amounts of antibody. Positive correlations

merely imply that animals that gave higher-than-average responses in the first test gave higher-than-average responses in the second test.

So far little work has been done to measure the plasma IgA activity against third-stage larvae of *T. circumcincta* in naturally infected lambs. . The present result suggests that IgA activity against third-stage larvae as a possible marker of resistance to nematode infection in sheep could be used rather than forth-stage larvae but more research is needed. The experimental results did not reveal any correlations between faecal egg counts or IgA activity against third-stage larvae. In contrast animals with increased IgA activity against fourth-stage larvae had lower faecal egg counts (Strain et al. 2002). Therefore IgA activity against fourth-stage larvae is probably a better marker of nematode resistance than IgA activity against third-stage larvae.

Other results reported weak correlations between IgA responses to third ($r = -0.155$) and fourth-stage ($r = -0.176$) larval extracts and egg counts (Henderson 2002) . The reasons for the differences are unknown but may be due to differences in exposure to nematode infection or nutrition. Unfortunately the results came from lambs on commercial farms and could not be investigated further. However, further trials considering different breeds and age groups of lambs at different periods of years at different environmental conditions should be considered.

There were no significant correlations between weight gain at 24 weeks old lambs and IgA activity during 2001, 2002 and 2003. There appear to be no previous published studies that have reported the relationship between IgA activity and growth rate.

More research is necessary to examine the relationship between nematode resistance and IgA activity against third-stage larvae. Further experiment would require necropsy of large numbers of naturally infected animals.

CHAPTER SIX

IDENTIFICATION OF QUANTITATIVE TRAIT LOCI INFLUENCING TRAITS RELATED TO IgA ACTIVITY AND FAECAL EGG COUNTS

6.1 Introduction

The control of nematode infection in ruminants relies mainly on the management of grazing and use of anthelmintic agents. However, grazing management systems are often impractical and expensive to implement, whereas frequent use of anthelmintics leads to problems such as rising resistance of parasites to drugs and increasing public concern about chemical residues in animal products and the environment (Sangster 1999).

Breeding sheep for increased nematode resistance would reduce the cost of anthelmintics and minimise the effect of internal nematodes on production. The difficulty of including nematode resistance in breeding programmes is mainly due to the difficulty of measuring nematode resistance itself. Thus, nematode resistance is recorded and included in breeding programmes through correlated traits; the most widely used being faecal egg count (FEC). This trait is costly to measure and can be misleading if anthelmintics have been given (Dominik 2005).

There are various other indicator traits that may be used to assess genetic resistance. In addition to faecal egg counts these indicator traits include immunoglobulin A (IgA) activity, eosinophil counts, pepsinogen activity and fructosamine concentration (Stear et al. 1995d;Stear et al. 1999a;Strain et al. 2002). One approach for the selection of individuals with superior resistance to GI nematodes is identification of the genomic loci responsible for the genetic variation in host (Dominik 2005). If resistance is under the control of many genes, all with small effects (polygenic), it is unlikely that a single genetic marker will be valuable in identifying resistant animals. However, if resistance is due to the effect of a single gene or the actions of only a few genes of moderate or large effect, a suitable marker is more likely to be detectable (Kahn et al. 2003).

The mapping of Quantitative Trait Loci (QTL) is the first step towards the identification of genes and causal polymorphisms for traits of importance in agriculture and human medicine (Seaton et al. 2002).

A number of studies have identified QTLs for resistance to gastrointestinal nematodes in sheep (Beh et al. 2002;Coltman et al. 2001a;Diez-Tascon et al. 2002;Janssen et al. 2002). In addition, research into nematode resistance has also been undertaken in mouse models and serves as a model for internal nematode resistance in sheep (Behnke et al. 2003).

Divergent sheep selection lines resistant or susceptible to nematode parasites were used to find that a QTL for resistance was localized in chromosome 3, and mapped to about a 5 cM region (Diez-Tascon et al. 2002). A gene located in this region codes for interferon gamma (IFN-γ) and is a putative candidate gene for resistance to nematode parasites (Diez-Tascon et al. 2002). In Australia (Beh et al. 2002) found a QTL for resistance to *T. colubriformis* in Merino sheep on chromosome 6.

Statistical analysis in Rhonschaf sheep showed significant associations between faecal egg count and the markers *OarCp73*, *DYMS1* and *BM1815* (Janssen et al. 2002). The *DYA* gene (belonging to the class IIb subregion of the major histocompatibility complex MHC) is closely linked to the microsatellite *DYMS1*, and is a possible candidate gene for resistance to *T. circumcincta* and *Haemonchus contortus* in sheep (Buitkamp et al. 1996;Janssen et al. 2002).

The *DRB1* locus lies within a QTL region on chromosome 20 which is a putative candidate due to its role in immunity (Zinkernagel and Doherty 1979). Polymorphisms at the *DRB1* locus of the MHC Class II are associated with resistance to *T. circumcincta* in Scottish

Blackface sheep (Schwaiger *et al.*, 1995). In particular 6-month old animals with the G2 allele had FEC 58 times lower then animals with the most common (I) allele.

The purpose of this chapter is to firstly identify QTL associated with nematode resistance which segregate in Scottish Blackface lambs using the FEC and immunoglobulin A (IgA) activity as indicator traits for host resistance and response to infection. Secondly it is to demonstrate the relationship between MHC class II DRB1 microsatellite polymorphism and allele sequences.

6.2 Material and Methods

6.2.1 Animals

A total of 789 straightbred Scottish Blackface lambs, comprising 9 half-sib families ranging from 23-141 individuals, were studied over a three-year period (2001-2003) and all were of known parentage. The complete pedigree contained 4847 animals with records dating back to 1986.

The lambs were born outside and were continually exposed to natural mixed nematode infection by grazing. Lambs were kept in two groups each year with the group being representative of the field grazed. Husbandry procedures followed standard commercial practice.

6.2.2 Parasitological methods

The parasitological methods have been described in Chapter two, section 2.1. Briefly faecal egg counts were made according to a modified McMaster method with saturated salt solution. Each sample was counted on four separate McMaster chambers and each egg counted

represented 12.5 eggs per gram. These counts will be referred to as epg1, epg2, epg3 and epg4.

6.2.3 ELISA assays

The ELISA methods for detection of IgA activity in third-stage larvae have been described in Chapter 2 section 2.2.3.

6.2.4 Molecular biology

The experimental procedures for DNA extraction and cloning and sequencing of MHC Class II *DRBI* have been described in Chapter two.

6.2.5 Genotyping and map construction

All animals were genotyped using microsatellite markers. This typing was carried out by a commercial company Agresearch Ltd in New Zealand using DNA supplied by us. Eight chromosomes were examined; they were chromosomes 1, 2, 3, 5, 14, 18, 20 and 21. These regions were chosen because of previous reports of QTL for nematode resistance (chromosomes 3 and 20) and lamb performance traits such as growth rate or meat quality. Each region contained between 9 and 34 markers. All sires were genotyped for all markers across each region. The markers used for specific chromosomes have been described in the figures 6.1 and 6.2. Offspring were subsequently genotyped for markers which were heterozygous in their sire. In total 139 markers were genotyped. Relative marker locations were established by creating a linkage map (Davies et al. 2005) for each chromosome using Cri-map (Green et al. 1990).

6.2.6 Data Analysis

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

For the QTL analysis, the traits analysed were IgA activity, FEC at weeks 16, 20 and 24 for Strongyles as well as an average animal effect that is described below. A restricted maximum likelihood algorithm, ASREML (Gilmour et al. 1996) fitting an animal repeatability model (i.e. ignoring genetic effects), was used to create an average effect for each animal for Strongyles FECs. This effect was an average effect across time as FEC was measured at 3 time points. This animal model also fitted fixed effects: year, management group, sex, type (twin or single) and day of birth (fitted as continuous effect). The average effect was calculated from the transformed FEC data.

Heritability estimates were calculated using ASREML (Gilmour et al. 1996). An animal model, including all known pedigree relationships (4847 animals), was fitted. This included the fixed effects of year, management group, sex, type (twin or single) and day of birth (fitted as a continuous effect).

6.2.7 Estimation of QTL Position

QTL analyses were performed using regression techniques implemented by QTL Express (Seaton et al. 2002). Briefly we used a two-step procedure for QTL mapping, by firstly determining the Identity-By-Descent (IBD) probabilities at specific chromosomal locations

from multiple marker data, and secondly fitting a statistical model to the observations and IBD coefficients. The probability of inheriting a particular sire chromosome at a particular position was calculated for each offspring at 1cM intervals (Knott et al. 1996). Phenotypes were then regressed upon the conditional probability that a particular haplotype is inherited from the sire, along each chromosome, fitting fixed effects of year, sex, litter size, management group and day of birth (fitted as a covariate). For each regression an F-ratio of the full model including the inheritance probability versus the same model without the inheritance probability was calculated across families, the location of the QTL was indicated by the largest F-value.

6.2.8 Significance Thresholds

The 5% chromosome-wide threshold was determined for each chromosome by permutation testing (1000 permutations) (Churchill and Doerge 1994). A 5% genome-wide threshold was then obtained by applying the Bonferroni correction (Knott et al. 1996)

$$P_{genome\text{-}wide} = 1 - (1 - p_{chromosome\text{-}wide})^{n}$$

Where n is the number of chromosomes. The genome-wide threshold is based on the assumption that by chance 0.05 significant results would be expected per genome analysis.

6.2.9 Confidence Intervals

For each QTL estimate that was significant at the 5% chromosome-wide level confidence intervals were calculated using the bootstrap method (Visscher et al. 1996). 1000 samples with replacement were used to estimate 95% confidence intervals. Bootstrap samples were created by sampling with replacement N individual observations. An observation consists of a marker genotype and phenotype.

6.2.10 Size of QTL Effects

The proportion of phenotypic variance explained by the QTL was calculated using:

Phenotypic proportion = 4 (1- MS_{full} / $MS_{reduced}$)

Where MS is the residual mean square from the regression analysis (Knott et al. 1996). By dividing this phenotypic value by the heritability, estimated using ASREML. The proportion of genetic variance explained by QTL can be calculated. As these results came from a half-sib analysis it was necessary to adjust the genetic proportion (GP) value to account for the proportional reduction in phenotypic variance expressed within sire families:

Adjusted GP = GP (1 - h^2 / 4)

The resulting value is an estimate of the size of the effect of the QTL, i.e. the proportion of total additive genetic variance that is explained by the QTL.

6.3 Results

Table 6.1 demonstrates the microsatellite polymorphism of the second exon of the MHC-class II DRB1, their length and sequences. A total of 19 different alleles were identified and 17 alleles could be distinguished on the basis of their length (Table 6.1). Sequence analysis of the DNA samples confirmed new sequences of the exon 2 of the DRB1 gene associated with microsatellite lengths of 498bp, 500bp, 512bp, 568bp and 572bp. However, some of the microsatellite alleles differ in their length, but appear to have the same sequences in the coding region (E&F), (I&K), (N&O) and (T, U and V). While a number of alleles have the same length of microsatellite but have different sequences in exon 2 (G1&G2) and (H1&H2).

Table 6.1 Sequences, Gene bank accession number and size of microsatellite alleles at *DRB* locus in naturally infected Scottish Black face lamb.

Microsatellite allele	Size (bp)	Sequence(Gene bank accession number)
A	416	ab017231
B	476	ab017228
C	480	ab017214
D	484	u00216
E	498	y035-1 *
F	500	y035-1 *
G1	512	y082-5 *
G2	512	ab017206
H1	522	af036562
H2	522	u00206
I	526	y10248
K	534	y10248
L	546	ab17230
M	556	ab17205
N	568	y072-2 *
O	572	y072-2 *
T	800	ab01720
U	800	ab01720
V	800	ab01720

* New sequences identified

Table 6.2 represents the Summary statistics for the traits of FEC and IgA activity in Strongyle FECs ranged from 0 to 5325. Strongyle FECs were considerably larger in August than in both September and October. IgA activity ranged from 0 to 1.24 with a standard deviation of 0.19.

Table 6.2 Summary statistics of the FEC and IgA activity

Trait	Age (weeks)	Number of observation	Mean	Maxi.*	Transformed means	Transformed standard deviation
IgA Activity	24	757	0.13	1.24	0.42	0.21
Strongyle FEC August	16	740	256	5325	4.85	1.28
Strongyle FEC September	20	721	288	2550	5.19	1.12
Strongyle FEC October	24	741	236	1700	5.12	1.02

* The minimum value for each trait was zero

Heritability estimates for all traits are shown in Table 6.3. They ranged from0.11 for epg in September to 0.50 for epg in August. The FEC heritability estimates are somewhat varied, as heritability estimates calculated with a restricted maximum likelihood algorithm are sensitive to data transformations. IgA activity appears to have a low heritability of 0.18 in this study. However there is a strong maternal effect.

Table 6.3 Heritabilities of FEC and IgA activity

Trait	Heritability (h^2)	Standard Error
Strongyle FEC August	0.50	0.12
Strongyle FEC September	0.11	0.07
Strongyle FEC October	0.21	0.09
Strongyle Average Animal Effect	0.23	0.09
IgA Activity	0.18	0.09

Significant QTL are shown in Table 6.4. The QTL analysis found putative QTL on chromosomes 3 and 20. These were associated with both Strongyle FEC traits and IgA. Chromosome 3 indicated a QTL associated with Strongyle FEC average animal effect at 150 cM. This QTL was significant at the 5% chromosome-wide threshold (Table 6.3).

Table 6.4 QTL significant at 5% chromosome-wide significance level of FEC and IgA activity

Trait	Chromosome	Position (cM)	F ratio	5% chromosome-wide Threshold	5% Genome-wide Threshold	95% Confidence Interval
IgA Activity	3	118	2.48	2.48	2.96	36-189.5
Strongyle Average	3	150	2.59	2.44	2.96	0-205
Animal Effect Strongyle FEC October	20	10	2.64	2.44	2.96	0-59
IgA Activity	20	40	2.90	2.45	2.96	1-65

The QTL accounted for 37% of the genetic variance (Table 6.5).

Table 6.5 Proportions of variation attributable to QTL effect of FEC and IgA activity

Trait	Chromosome	Heritability	Phenotypic proportion	Genetic proportion
Strongyle FEC October	3	0.21	0.08	0.37
Strongyle Average Animal Effect	20	0.23	0.08	0.31
IgA Activity	20	0.18	0.10	0.51
IgA Activity	3	0.18	0.08	0.41

The position 118cM on chromosome 3 (Fig.6.1) had a significant QTL for IgA activity at the 5% chromosome wide threshold (Table 6.4). The size of the QTL effect was 41% (Table 6.3). On chromosome 20 a QTL was observed for Strongyle FEC October at 10cM. This QTL occurred at or the inverted MHC region and it accounted for 31% of the genetic variance. Indications of a QTL were observed for IgA activity at 40cM on the same chromosome (Fig. 6.2). This QTL was significant at the 5% chromosome-wide threshold (Table 6.4) and the size of the QTL effect was 0.51 (Table 6.5). This QTL is in the region that contains the classical MHC loci.

Fig. 6.1 QTL contour plot of chromosome 3 for FEC and IgA activity

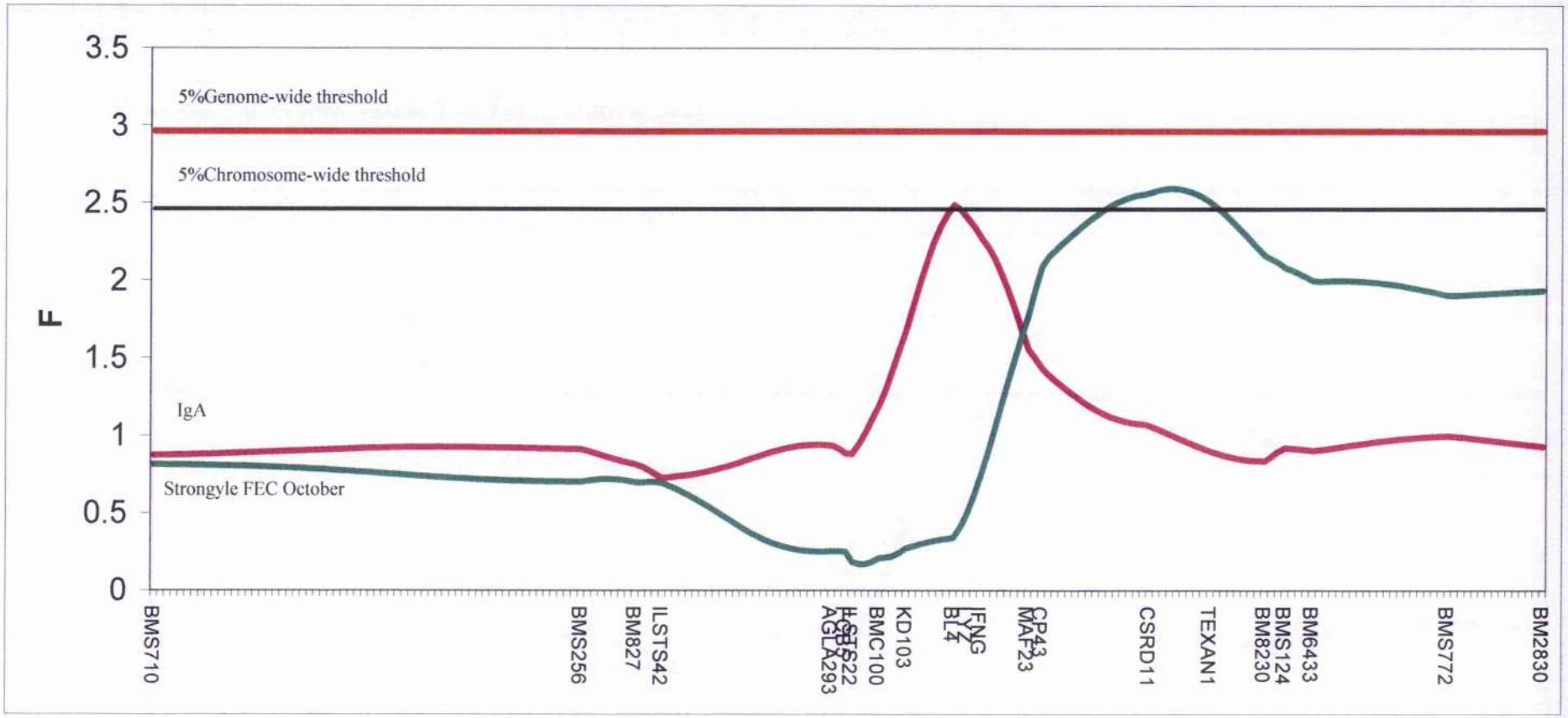
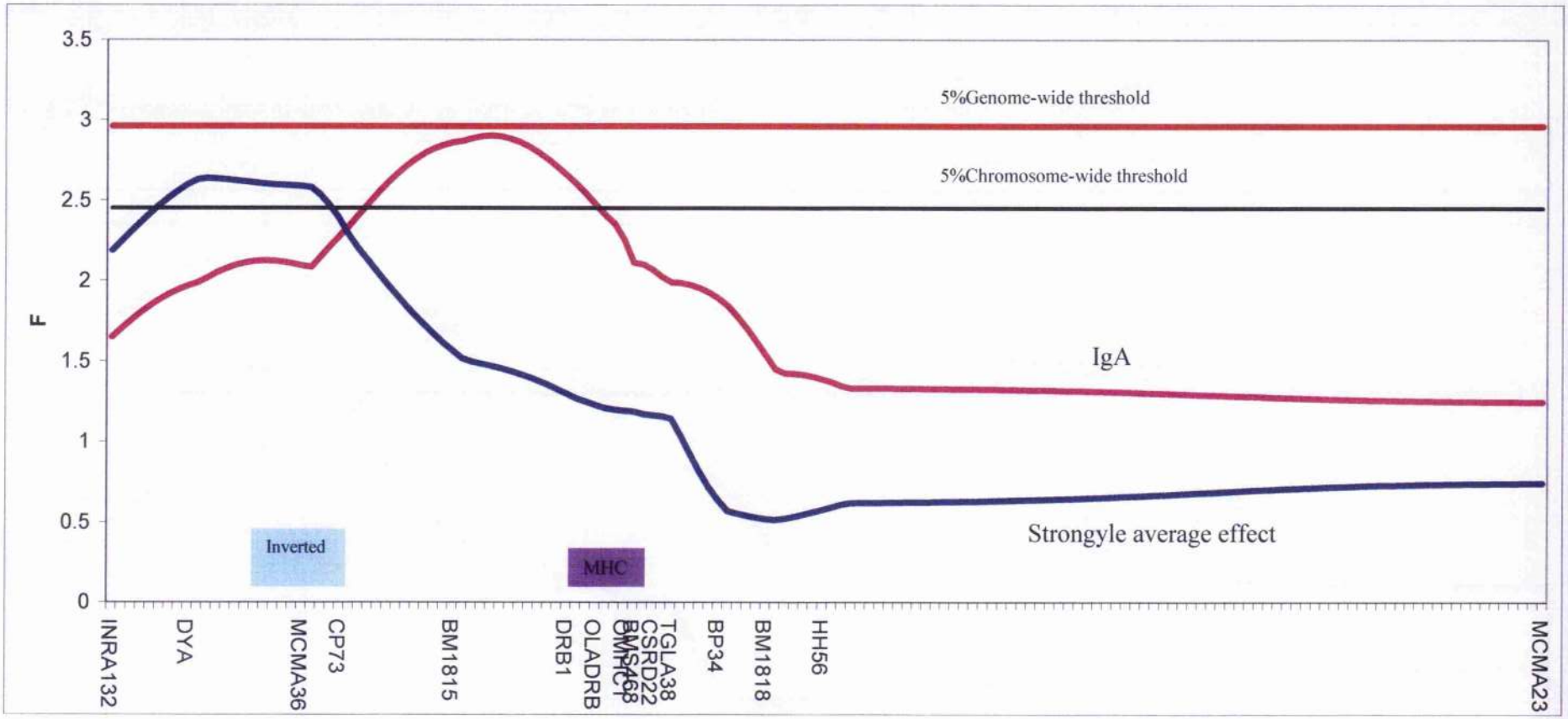


Fig. 6.2 QTL contour plot of chromosome 20 for FEC and IgA activity



6.4 Discussion

In this study three new sequences have been identified at the MHC class II *DRB1* gene of Scottish Blackface sheep. The sequencing of the MHC class II *DRB1* alleles is an essential step for understanding the genetic basis of resistance to gastrointestinal nematode infection and other diseases that affect sheep, as MHC polymorphism has been related to genetic resistance to *T. circumcincta* (Schwaiger et al. 1995). The new sequences in this chapter are Y035-1, Y082-5 and Y072-2, which were associated with microsatellite lengths of 498bp-500bp, 512bp and 568bp-572bp respectively.

There was evidence for QTL on 2 chromosomes for various FEC traits and IgA activity. The QTL identified on these two chromosomes are close to regions linked to immune function. The QTL on chromosome 3 associated with IgA activity is very close to that for Interferon gamma (IFN-γ). This could be a possible candidate gene for nematode resistance. IFN-γ has an important role in the regulation of the immune response to pathogens (Urban et al. 1996;Wakelin 1996). IFN-γ is a cytokine which is secreted by Th1 immune cells. IFN-γ activates macrophages which then become more phagocytic i.e. they are more capable of killing intracellular pathogens and have increased ability to present antigens. Previous evidence for QTL associated with parasitic infection on chromosome 3 in the region of IFN-γ has been reported in several studies. Paterson et al (2001) suggested a QTL in the interval IFN-γ – BMS1617 for a multispecies parasite challenge in Romney sheep divergent selection lines. Evidence for a QTL associated with *T. circumcincta* was reported in Soay sheep, again close to IFN-γ (Coltman et al. 2001a) and a QTL for *Trichostrongylus colubriformis* was observed in Merino sheep divergent selection lines in the IFN-γ region (Beh et al. 2002). These QTL are very close in position to the QTL identified in this study and they come from diverse breeds that were challenged with different species of nematodes. QTL that occur in

different breeds and confer resistance to multiple species of parasites are likely to be of widespread use in animal breeding.

In sheep, the Major Histocompatibility Complex (MHC) is found in 2 regions of chromosome 20. The QTL found on chromosome 20 in this study are very close to the two MHC regions. These regions could contain possible candidate genes as the MHC consists of a group of closely linked genes involved in antigen presentation to the vertebrate immune system. The primary immunological function of MHC molecules is to bind and 'present' antigenic peptides on the surfaces of cells for recognition by the antigen-specific T-cell receptors of lymphocytes. The MHC region has been suggested as a region for putative QTL in previous studies (Buitkamp et al. 1996;Schwaiger et al. 1995;Stear et al. 1996a).

Significant associations between microsatellite polymorphism in *DRB1* and FEC have also been observed in primitive Heather head sheep (Charon et al. 2002). Three QTL were reported in a Rhonschaf flock for haematocrit level (CP73), IgL level (DYMS1) and FEC (BM1815) after an artificial challenge with *H. contortus* (Janssen et al. 2002). This result for a similar chromosomal region from a different breed with a different parasite and different trait measurements supports the view that one or more QTL for nematode resistance exist within or around the ovine MHC.

Sizes of the QTL effects calculated in this study are very large for some of the traits. This may be due to the fact that the heritability estimates are quite low and also are very sensitive to the data transformation used; therefore the estimate of the proportion of genetic variability may be less precise than the phenotypic estimate. As it was necessary to investigate the effects of the transformations on the heritability estimates, the effect on QTL locations was also considered. However, the regression techniques used in QTL mapping were not sensitive to

the transformation. In particular, the positions of the QTL were essentially identical irrespective of the transformation used, and the F ratio was only slightly affected. The relatively imprecise estimate of the genetic variance could have led us to overestimate the proportion of genetic variance accounted for by the QTL.

In conclusion, this study has provided evidence for QTL linked to parasitic infection and immune response on two chromosomes. These chromosomes have potential candidate genes/regions that have been previously shown to influence immune function. The results of this study confirm that parasite resistance is under genetic control and provides results that could help to select sheep for increased resistance to parasitic infection.

CHAPTER SEVEN

GENERAL DISCUSSION

Nematode infection threatens the health and welfare of livestock and compromises the efficiency of livestock production. Nematodes are possibly the major disease challenge facing ruminants (Perry and Randolph 1999). They are ubiquitous and have a major impact on all production areas (Urquhart et al. 1996). Essentially all grazing animals are infected.

Many different species of nematodes are pathogenic and they differ in their natural history, epidemiology and pathology (Urquhart et al. 1996). The mixture of species differs among different climates and much unnecessary confusion has been caused by the incautious extrapolation of results from one production system to another. In the UK, the dominant nematode is *Teladorsagia circumcincta* but most animals are infected with a mixture of species, including Nematodirus spp., Cooperia spp. and Trichostrongylus spp.

Nematodes cause disease but perhaps their major economic impact is the reduction in growth of young lambs (Coop et al. 1977). The severity of disease and the loss of production depend upon the intensity of infection, immunity of the host and its relative nutritional status (Coop and Kyriazakis 2000;Stear et al. 2003). The intensity of infection is influenced by the weather and management factors such as stocking rate, frequency of anthelmintic treatment and the number of times animals are moved to less contaminated pastures(Coop and Kyriazakis 2000). Host immunity is strongly influenced by genetic factors but also depends upon age, nutrition and history of exposure. Relative nutritional status depends not only on past and current diet, especially protein intake (Coop and Kyriazakis 2000) but also upon the intensity of infection.

Efficient and healthy livestock production demands the control of nematode infection. Current control measures rely upon anthelmintic treatment but are threatened by the widespread evolution of drug-resistance in parasite populations (Bartley et al. 2004). A

variety of potential control methods have been advocated including vaccination (Knox et al. 2003), supplementary feeding (Coop and Kyriazakis 2000), and biological methods such as the use of nematophagous fungi and species of grass that prevent nematode infection (Waller and Faedo 1996). Selective breeding of sheep for resistance to nematode production is an attractive, sustainable method of nematode control (Bisset et al. 2001;Eady et al. 2002;Stear et al. 2001a).

The exploitation of host genetic variation using faecal egg count (FEC) in commercial sheep breeding programmes, is a well-established breeding practice in New Zealand ('WormFEC') and Australia ('Nemesis'). Research in the UK has shown that genetic correlations between the faecal egg counts arising from different nematode taxa are close to 0.5 (Stear, personal communication). A positive genetic correlation of 0.5 suggests that selection for resistance to nematodes confers resistance to other species, not necessarily present at the time of sampling. Also, in the periparturient ewe, FEC is moderately heritable (Bishop and Stear 2001b;Morris 1998;Woolaston 1992) and genetically correlated with resistance in the lamb (Morris 1998). This means that additional benefits accrue from reduced pasture contamination and decreased larval challenge with indirect benefits on health and performance (Bishop and Stear 2003).

In Australia, the different mix of nematodes means that FEC are a better marker of nematode resistance than in the UK. Egg counts show a linear relationship with the number of adult nematodes in Australian conditions (Roberts and Swan 1981). A similar linear relationship is assumed to exist in New Zealand but detailed information is lacking. The relationship is more complex in Scottish lambs (Bishop and Stear 2000b). Lambs infected with high numbers of adult nematodes can have lower egg counts than more lightly infected contemporaries (Stear et al. 1998). One consequence of this complex relationship in Scottish sheep is that a selection scheme based solely on egg counts will make slower progress than comparable schemes

elsewhere. Even so, alternatives to FEC are routinely used in New Zealand (parasite specific antibody responses) and in Australia (haematocrit values).

There are several markers for nematode resistance in naturally infected Scottish sheep that may offer greater responses to selection. They also appear cheaper and more convenient for farmers than egg counts. These markers are both phenotypic and genetic. The phenotypic markers include plasma IgA activity (Strain et al. 2002), pepsinogenaemia (Stear et al. 1999a), fructosamine concentrations in the plasma (Stear et al. 2001b) and eosinophilia (Stear et al. 2002a). The genetic markers include the major histocompatibility complex (Schwaiger et al. 1995;Stear et al. 2005b) and the interferon gamma region (Coltman et al. 2001c).

Phenotypic indicators have been investigated on a number of occasions and consistently show moderate to strong heritabilities in commercial conditions. For example, IgG specific to *Trichostrongylus colubriformis* (Douch et al. 1995b); plasma IgA activity against larval stages of *T. circumcincta* (Strain et al. 2002), pepsinogenaemia (Stear et al. 1999a), fructosamine concentrations in the plasma (Stear et al. 2001c) and eosinophilia (Stear et al. 2002a). (Davies et al. 2005) reported that these indicator traits were genetically correlated with worm size and fecundity.

Quantitative trait loci (QTL) for nematode resistance have been detected in New Zealand, Australia, Kenya, US and Europe, including UK, France, Italy and Spain. QTL for FEC occur on chromosome 1 for *T. colubriformis* (Beh et al. 2002;Diez-Tascon et al. 2002), chromosome 3 for *T. colubriformis* (Beh et al. 2002), and mixed natural infection in New Zealand (Paterson and Banks 2001), and the UK (Coltman et al. 2001b;Davies et al. 2005), chromosome 6 for *T. colubriformis* (Beh et al. 2002), chromosome 14 Nematodirus (Davies et

al. 2005), and chromosome 20 (mixed natural infection in the UK (Buitkamp et al. 1996;Schwaiger et al. 1995;Stear et al. 1996a;Stear et al. 2005b).

In summary, genetic variation in many aspects of host resistance to nematodes is well documented. Several phenotypic markers exist. There has been considerable success in QTL detection, and two QTL on chromosomes 3 and 20 have been confirmed in several independent studies. All these traits can be assayed from a single blood sample. If the blood sample taken is also used for further genetic and physiological screening (e.g. for resistance to scrapie, and potentially, for resistance to footrot), then multiple testing of blood samples may well prove to be the most economically efficient route to assess genetic resistance to several diseases.

The purpose of this thesis was to examine several of these markers in more detail. Chapters 3 and 4 examined faecal egg counts. Chapter 3 looked at the repeatability of egg counts from the same faecal sample and from samples taken at monthly intervals. The replicate counts from the same sample gave high repeatabilities but the samples taken four weeks apart were much more weakly correlated. Therefore multiple samples will improve the estimates of genetic merit but multiple samples may be difficult to obtain under commercial conditions.

Faecal egg counts generally but not always followed a negative binomial distribution. The results from chapter 4 suggested that the deviation from the negative binomial was a consequence of multiple species contributing to the egg count. Understanding the distribution of egg counts is important for parametric analyses that require specific known distributions The distribution of mean egg counts across populations was similar to a gamma distribution. Gamma distributions are overdispersed and this distribution indicates that most populations have relatively low mean counts but a small proportion of populations have relatively high

egg counts. Modern selection schemes will involve multiple farms and again knowledge of the underlying distribution is required for meaningful data analyses.

Chapter 5 looked at the distribution of IgA activity against third-stage larvae of *T. circumcincta*. Published results for IgA activity have mainly dealt with fourth-stage larvae. To obtain fourth-stage larvae it is necessary to sacrifice lambs and this is prohibitively expensive for large scale commercial testing. Third-stage larvae offer a much simpler alternative and the response to third and fourth-stage larvae are correlated (Sinski et al. 1995b). The results showed that the distribution of IgA activity was overdispersed but more work is necessary to determine the best-fitting distribution. Log-transformation offers a simple method for data analysis. There was no phenotypic relationship between IgA activity and faecal egg counts or growth rate. However, faecal egg counts are not a gold standard for parasite resistance as they show a complex relationship with worm number and fecundity (Bishop and Stear 2000b). More research is necessary to examine the relationship between nematode resistance and IgA activity against third-stage larvae. Perhaps the most suitable experiment would require necropsy of large numbers of naturally infected sheep.

The final chapter examined the *DRB1* locus of the major histocompatibility complex which has already been shown to be associated with resistance to nematode infection (Paterson et al. 1998;Schwaiger et al. 1995). The results showed that there were at least 14 alleles in the Scottish Blackface population. To our knowledge this is the first comprehensive description of variation at this important locus in any livestock population. Further research is now needed to determine the full length sequences of these alleles and to compare variation with other populations such as the human.

Linkage analysis demonstrated a relationship between IgA activity and a region on chromosome 20 close to *DRB1*. There was also a relationship between faecal egg count and a region on chromosome 20 that contained the other inverted part of the major histocompatibility complex. More research is needed to determine whether these regions contain the same or two distinct QTL. Further research should concentrate on fine-mapping these gene(s).

In conclusion, this thesis has examined some of the determinants of resistance to nematode infection at both the genetic and phenotypic levels. Selective breeding is already taking place on a small number of commercial farms. The research reported here will help to advance that process by providing better understanding of the genetic and phenotypic markers. The work detailed in this thesis has further increased our understanding of the complex host/parasite relationship, and has confirmed that selective breeding, using the various phenotypic and genetic markers studied, is possible.

Appendix

This SAS program provides means, variances and k values for the distribution of faecal egg counts.

```
filename aug2001 dde
'excel|L:\Clinical Studies\FAMP\Teladorsagia\EC\Egg
counts\bbepg130801.xls!r3c1:r242c11';

data onea;
 infile aug2001 missover;
 input lamb $ epg1 epg2 epg3 epg4 nem1 nem2 nem3 nem4 eim $ mon $;
 if epg1 = . then delete;
 epgaug = sum(epg1,epg2,epg3,epg4)/4;
epgaugct =(4*epgaug)/50;
 if mon = 'mon ' then mon = '1'; else mon ='0';
 if eim = 'eim ' then eim = '1'; else eim ='0';
run;

proc univariate plot normal;
 var epgaug ;

proc genmod;
  model epgaugct =/ dist = nb
            link = log lrci;
run;

filename sep2001 dde
'excel|L:\Clinical Studies\FAMP\Teladorsagia\EC\Egg
counts\bbepg100901.xls!r3c1:r221c11';

data oneb;
 infile sep2001 missover;
 input lamb $ epg1 epg2 epg3 epg4 nem1 nem2 nem3 nem4 eim $ mon $;
 if epg1 = . then delete;
 epgsep = sum(epg1,epg2,epg3,epg4)/4;
 if mon = 'mon ' then mon = '1'; else mon ='0';
 if eim = 'eim ' then eim = '1'; else eim ='0';
epgsepct =(4*epgsep)/50;
proc univariate plot normal;
 var epgsep;

proc genmod;
  model epgsepct =/ dist = nb
            link = log lrci;
run;

filename oct2001 dde
'excel|L:\Clinical Studies\FAMP\Teladorsagia\EC\Egg
counts\bbepg081001.xls!r3c1:r231c11';

data onec;
 infile oct2001 missover;
 input lamb $ epg1 epg2 epg3 epg4 nem1 nem2 nem3 nem4 eim S mon $;
```

```
 if epg1 = . then delete;
 epgoct = sum(epg1,epg2,epg3,epg4)/4;
 if mon = 'mon ' then mon = '1'; else mon ='0';
 if eim = 'eim ' then eim = '1'; else eim ='0';
 epgoctct =(4*epgoct)/50;
run;

proc univariate plot normal;
 var epgoct;

 proc genmod;
  model epgoctct =/ dist = nb
            link = log lrci;
run;
*/
filename aug2002 dde
'excel|L:\Clinical Studies\FAMP\Teladorsagia\EC\Egg
counts\bbepg130802.xls!r3c1:r252c11';

data twoa;
 infile aug2002 missover;
 input lamb $ epg1 epg2 epg3 epg4 nem1 nem2 nem3 nem4 eim $ mon $;
 if epg1 = . then delete;
 epgaug = sum(epg1,epg2,epg3,epg4)/4;
epgaugct =(4*epgaug)/50;
 if mon = 'mon ' then mon = '1'; else mon ='0';
 if eim = 'eim ' then eim = '1'; else eim ='0';
run;

proc univariate plot normal;
 var epgaug ;

proc genmod;
  model epgaugct =/ dist = nb
            link = log lrci;
run;

filename sep2002 dde
'excel|L:\Clinical Studies\FAMP\Teladorsagia\EC\Egg
counts\bbepg090902.xls!r3c1:r258c11';

data twob;
 infile sep2002 missover;
 input lamb $ epg1 epg2 epg3 epg4 nem1 nem2 nem3 nem4 eim $ mon $;
 if epg1 = . then delete;
 epgsep = sum(epg1,epg2,epg3,epg4)/4;
 if mon = 'mon ' then mon = '1'; else mon ='0';
 if eim = 'eim ' then eim = '1'; else eim ='0';
epgsepct =(4*epgsep)/50;
proc univariate plot normal;
 var epgsep;
```

```
proc genmod;
  model epgsept =/ dist = nb
            link = log lrci;
run;

filename oct2002 dde
'excel|L:\Clinical Studies\FAMP\Teladorsagia\EC\Egg
counts\bbepg071002.xls!r3c1:r264c11';

data twoc;
 infile oct2002 missover;
 input lamb $ epg1 epg2 epg3 epg4 nem1 nem2 nem3 nem4 eim $ mon $;
 if epg1 = . then delete;
 epgoct = sum(epg1,epg2,epg3,epg4)/4;
 if mon = 'mon ' then mon = '1'; else mon ='0';
 if eim = 'eim ' then eim = '1'; else eim ='0';
 epgoctct =(4*epgoct)/50;
run;

proc univariate plot normal;
 var epgoct;

 proc genmod;
  model epgoctct =/ dist = nb
            link = log lrci;
run;

filename aug2003 dde
'excel|L:\Clinical Studies\FAMP\Teladorsagia\EC\Egg
counts\bbepg180803.xls!r3c1:r256c11';

data threea;
 infile aug2003 missover;
 input lamb $ epg1 epg2 epg3 epg4 nem1 nem2 nem3 nem4 eim $ mon $;
 if epg1 = . then delete;
 epgaug = sum(epg1,epg2,epg3,epg4)/4;
epgaugct =(4*epgaug)/50;
 if mon = 'mon ' then mon = '1'; else mon ='0';
 if eim = 'eim ' then eim = '1'; else eim ='0';
run;

proc univariate plot normal;
 var epgaug ;

proc genmod;
  model epgaugct =/ dist = nb
            link = log lrci;
run;

filename sep2003 dde
'excel|L:\Clinical Studies\FAMP\Teladorsagia\EC\Egg
counts\bbepg150903.xls!r3c1:r259c11';
```

```
data threeb;
 infile sep2003 missover;
 input lamb $ epg1 epg2 epg3 epg4 nem1 nem2 nem3 nem4 eim $ mon $;
 if epg1 = . then delete;
 epgsep = sum(epg1,epg2,epg3,epg4)/4;
 if mon = 'mon ' then mon = '1'; else mon ='0';
 if eim = 'eim ' then eim = '1'; else eim ='0';
epgsepct =(4*epgsep)/50;

proc univariate plot normal;
 var epgsep;

proc genmod;
  model epgsepct =/ dist = nb
            link = log lrci;
run;

filename oct2003 dde
'excel|L:\Clinical Studies\FAMP\Teladorsagia\EC\Egg
counts\bbepg180803.xls!r1c1:r255c11';

data threec;
 infile oct2003 missover;
 input lamb $ epg1 epg2 epg3 epg4 nem1 nem2 nem3 nem4 eim $ mon $;
 if epg1 = . then delete;
 epgoct = sum(epg1,epg2,epg3,epg4)/4;
 if mon = 'mon ' then mon = '1'; else mon ='0';
 if eim = 'eim ' then eim = '1'; else eim ='0';
 epgoctct =(4*epgoct)/50;
run;

proc univariate plot normal;
 var epgoct;

 proc genmod;
  model epgoctct =/ dist = nb
            link = log lrci;
run;
```

List of References

Adams DB (1989) A preliminary evaluation of factors affecting an experimental system for vaccination and challenge with *Haemonchus contortus* in sheep. International Journal for Parasitology **19**:169-175

Albers GA, Gray GD, Piper LR, Barker JS, Le Jambre LF, Barger IA (1987) The genetics of resistance and resilience to *Haemonchus contortus* infection in young merino sheep. International Journal for Parasitology **17**:1355-1363

Altaif KI, Dargie JD (1978) Genetic resistance to helminths. Comparison of the development of Ostertagia circumcincta infections in Scottish Blackface sheep of different haemoglobin type. Research in Veterinary Science **24**:391-393

Amarante AF, Craig TM, Ramsey WS, Davis SK, Bazer FW (1999) Nematode burdens and cellular responses in the abomasal mucosa and blood of Florida Native, Rambouillet and crossbreed lambs. Veterinary Parasitology **80**:311-324

Anderson N, Hansky J, Titchen DA (1985) Effects on plasma pepsinogen, gastrin and pancreatic polypeptide of Ostertagia spp. transferred directly into the abomasum of sheep. International Journal for Parasitology **15**:159-165

Anderson RM (1978) The regulation of host population growth by parasitic species. Parasitology **76**:119-157

Anderson RM, Gordon DM, Crawley MJ, Hassell MP (1982) Variability in the abundance of animal and plant species. Nature **296**:245-248

Anderson RM, May RM (1985) Helminth infections of humans: mathematical models, population dynamics, and control. Advances in Parasitology **24**:1-101

Armour J, Al Saqur IM, Bairden K, Duncan JL, Urquhart GM (1980) Parasitic bronchitis and ostertagiasis on aftermath grazing. Veterinary Record **106**:184-185

Armour J, Coop RL (1991) Pathogenesis and control of gastrointestinal helminthiasis. In: Martin WB, Aitken ID (eds) Blackwell Scientific Publications, Oxford, pp 122-30

Armour J, Jarrett WFH, Jennings FW (1966) Experimental *Ostertagia circumcincta* infections in sheep: Development and pathogenesis of a single infection. American Journal of Veterinary Research **27**:1267-1278

Baker DG, Gershwin LJ (1993) Inverse relationship between IgE and worm burdens in cattle infected with *Ostertagia ostertagi*. Veterinary Parasitology **47**:87-97

Baker RL (1995) Genetics of disease resistance in small ruminants in Africa. In: Gray GD, Woolaston RR, Eaton BT (eds) Canberra, pp 119-38

Balic A, Bowles VM, Meeusen ENT (2000) The Immunobiology of gastrointestinal nematode infections in ruminants. Advances in Parasitology **45**:181-241

Barger I (1997) Control by management. Veterinary Parasitology **72**:493-500

Barger IA (1985) The statistical distribution of trichostrongylid nematodes in grazing lambs. International Journal for Parasitology **15**:645-649

Barger IA (1989) Genetic resistance of hosts and its influence on epidemiology. Veterinary Parasitology **32**:21-35

Barger IA (1993) Influence of sex and reproductive status on susceptibility of ruminants to nematode parasitism. International Journal for Parasitology **23**:463-469

Barger IA (1996) Prospects for integration of novel parasite control options into grazing systems. International Journal for Parasitology **26**:1001-1007

Barnes EH, Dobson RJ, Barger IA (1995) Worm control and anthelmintic resistance: adventures with a model. Parasitology Today **11**:56-63

Bartley DJ, Jackson F, Jackson E, Sargison N (2004) Characterisation of two triple resistant field isolates of Teladorsagia from Scottish lowland sheep farms. Veterinary Parasitology **123**:189-199

Beh KJ, Hulme DJ, Callaghan MJ, Leish Z, Lenane I, Windon RG, Maddox JF (2002) A genome scan for quantitative trait loci affecting resistance to Trichostrongylus colubriformis in sheep. Animal Genetics **33**:97-106

Beh KJ, Maddox JF (1996) Prospects for development of genetic markers for resistance to gastrointestinal parasite infection in sheep. International Journal for Parasitology **26**:879-897

Behnke JM, Iraqi F, Menge D, Baker RL, Gibson J, Wakelin D (2003) Chasing the genes that control resistance to gastrointestinal nematodes. Journal of Helminthology **77**:99-110

Bird J, Shulaw WP, Pope WF, Bremer CA (2001) Control of anthelmintic resistant endoparasites in a commercial sheep flock through parasite community replacement. Veterinary Parasitology **97**:219-225

Bishop SC, Bairden K, McKellar QA, Park M, Stear MJ (1996) Genetic parameters for faecal egg count following mixed, natural, predominantly *Ostertagia circumcincta* infection and relationships with liveweight in young lambs. Animal Science **63**:423-428

Bishop SC, Stear MJ (1999) Genetic and epidemiological relationships between productivity and disease resistance: gastro-intestinal parasite infection in growing lambs. Animal Science **69**:515-524

Bishop SC, Stear MJ (2000a) Genetic control of resistance to gastrointestinal parasites in hill sheep. Personal communication

Bishop SC, Stear MJ (2000b) The use of a gamma-type function to assess the relationship between the number of adult *Teladorsagia circumcincta* and total egg output. Parasitology **121**:435-440

Bishop SC, Stear MJ (2001a) Genetic improvement of resistance to nematodes in terminal sire sheep. Personal communication

Bishop SC, Stear MJ (2001b) Inheritance of faecal egg counts during early lactation in Scottish Blackface ewes facing mixed, natural nematode infections. Animal Science **73**:389-395

Bishop SC, Stear MJ (2003) Modeling of host genetics and resistance to infectious diseases: Understanding and controlling nematode infections. Veterinary Parasitology **115**:147-166

Bisset SA, Morris CA (1996) Feasibility and implications of breeding sheep for resilience to nematode challenge. International Journal for Parasitology **26**:857-868

Bisset SA, Morris CA, McEwan JC, Vlassoff A (2001) Breeding sheep in New Zealand that are less reliant on anthelmintics to maintain health and productivity. New Zealand Veterinary Journal **49**:236-246

Bisset SA, Vlassoff A, Morris CA, Southey BR, Baker RL, Parker AGH (1992) Heritability of and genetic correlations among faecal egg counts and productivity traits in Romney sheep. New Zealand Journal of Agricultural Research **35**:51-58

Blaxter M, Dorris M, De Ley P (2000) Patterns and processes in the evolution of animal parasitic nematodes. Nematology **2**:43-55

Bliss CI, Fisher RA (1953) Fitting the negative binomial distribution to biological data. Biometrics **9**:176-200

Boag B, Hackett CA, Topham PB (1992) The use of Taylor's power law to describe the aggregated distribution of gastro-intestinal nematodes of sheep. International Journal for Parasitology **22**:267-270

Boag B, Thomas RJ (1970) The development and survival of free-living stages of *Trichostrongylus colubriformis* and *Ostertagia circumcincta* on pasture. Research in Veterinary Science **11**:380-381

Boag B, Thomas RJ (1971) Epidemiological studies on gastro-intestinal nematode parasites of sheep. Infection patterns on clean and autumn-contaminated pasture. Research in Veterinary Science **12**:132-139

Boag B, Thomas RJ (1973) Epidemiological studies on gastro-intestinal nematode parasites of sheep. The control of infection in lambs on clean pasture. Research in Veterinary Science **14**:11-20

Boag B, Thomas RJ (1977) Epidemiological studies on gastro-intestinal nematode parasites of sheep: the seasonal number of generations and succession of species. Research in Veterinary Science **22**:62-67

Bodmer JG, Marsh SG, Albert E (1990) Nomenclature for factors of the HLA system, 1989. Immunology Today **11**:3-10

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

Buitkamp J, Filmether P, Stear MJ, Epplen JT (1996) Class I and class II major histocompatibility complex alleles are associated with faecal egg counts following natural, predominantly *Ostertagia circumcincta* infection. Parasitology Research **82**:693-696

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

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics **138**:963-971

Claerebout E, Vercruysse J (2000) The immune response and the evaluation of acquired immunity against gastrointestinal nematodes in cattle: a review. Parasitology **120**:S25-S42

Clarkson MJ, Winter AC (1997) A Handbook for the sheep clinician. University of Liverpool Press.,

Colditz IG, Watson DL, Gray GD, Eady SJ (1996) Some relationships between age, immune responsiveness and resistance to parasites in ruminants. International Journal for Parasitology **26**:869-877

Coles GC (1998) Drug-resistant parasites of sheep: An emerging problem in Britain? Parasitology Today **14**:86-88

Coltman DW, Pilkington J, Kruuk LEB, Wilson K, Pemberton JM (2001a) Positive genetic correlation between parasite resistance and body size in a free-living ungulate population. Evolution **55**:2116-2125

Coltman DW, Wilson K, Pilkington J, Stear MJ, Pemberton JM (2001b) A microsatellite polymorphism in the gamma interferon gene is associated with resistance to gastrointestinal nematodes in a naturally-parasitized population of Soay sheep. Parasitology **122**:571-582

Coltman DW, Wilson K, Pilkington JG, Stear MJ, Pemberton JM (2001c) A microsatellite polymorphism in the gamma interferon gene is associated with resistance to gastrointestinal nematodes in a naturally-parasitized population of Soay sheep. Parasitology **122**:571-582

Coop RL, Graham RB, Jackson F, Wright SE, Angus KW (1985) Effect of experimental *Ostertagia circumcincta* infection on the performance of grazing lambs. Research in Veterinary Science **38**:282-287

Coop RL, Holmes PH (1996) Nutrition and parasite interaction. International Journal for Parasitology **26**:951-962

Coop RL, Huntley JF, Smith WD (1995) Effect of dietary-protein supplementation on the development of immunity to *Ostertagia circumcincta* in growing lambs. Research in Veterinary Science **59**:24-29

Coop RL, Kyriazakis I (1999) Nutrition-parasite interaction. Veterinary Parasitology **84**:187-204

Coop RL, Kyriazakis I (2000) Influence of host nutrition on the development and consequences of parasitism in livestock. Parasitology Today **17**:325-330

Coop RL, Sykes AR, Angus KW (1977) The effect of a daily intake of Ostertagia circumcincta larvae on body weight, food intake and concentration of serum constituents in sheep. Research in Veterinary Science **23**:76-83

Coop RL, Sykes AR, Angus KW (1982) The effect of three levels of *Ostertagia circumcincta* larvae on growth rate, food intake and body composition of growing lambs. Journal of Agricultural Science **98**:247-255

Cornwell RL (1975) The pattern of infection with gastro-intestinal nematodes in young fattening lambs at pasture. Research in Veterinary Science **18**:1-5

Coyne CP, Brake D (2001) Characterisation of *Haemonchus contortus*-derived cell populations propagated in vitro in a tissue culture environment and their potential to induce protective immunity in sheep. International Journal for Parasitology **31**:359-376

Crawford A, McEwan JC, Dodds KG, Wright CS, Bisset SA, Macdonald PA, Knowler KJJ, Greer GJ, Green RS, Shaw RJ, Paterson KA, Cuthbertson RP, Vlassoff A, Squire DR, West CJ, Phua SH (1997) Resistance to nematode parasites in sheep: How important are the MHc genes? Proceedings of the Association for the Advancement of Animal Breeding and Genetics **12**:58-62

D'Agostino RB, Stephens MA (1986) Goodness-of-fit techniques. Marcel Dekker, New York

Dalton JP, Mulcahy G (2001) Parasite vaccines--a reality?. Veterinary Parasitology **98**:149-167

Davies G, Stear MJ, Bishop SC (2005) Genetic relationships between indicator traits and nematode parasite infection levels in 6-month-old lambs. Animal Science **80**:143-150

Diez-Tascon C, McDonald PA, Dodds KG, McEwan JC, Crawford AM (2002) A screen of chromosome 1 for QTL affecting nematode resistance in ovine outcross population. Proceedings of the 7th World Congress on Genetics Applied to Livestock Production,

Dineen JK, Gregg P, Lascelles AK (1978) The response of lambs to vaccination at weaning with irradiated *Trichostrongylus colubriformis* larvae: segregation into 'responders' and 'non-responders'. International Journal for Parasitology **8**:59-63

Dineen JK, Gregg P, Windon RG, Donald AD, Kelly JD (1977) The role of immunologically specific and non-specific components of resistance in cross-protection to intestinal nematodes. International Journal for Parasitology **7**:211-215

Dobson C (1964) Host edocrine interactions with nematode infections. I. Effect of sex, gonadectomy and thyroidectomy in lambs. Experimental Parasitology **15**:200-212

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

Dominik S (2005) Quantitative trait loci for internal nematode resistance in sheep: a review. Genetics Selection Evolution **37**:S83-S96

Donald AD (1968) Population studies on the infective stage of some nematode parasites of sheep. III. The distribution of strongyloid egg output in flocks of sheep. Parasitology **58**:951-960

Douch PG, Green RS, Morris CA, Hickey SM (1995a) Genetic factors affecting antibody responses to four species of nematode parasite in Romney ewe lambs. International Journal for Parasitology **25**:823-828

Douch PG, Green RS, Morris CA, McEewan JC, Windon RG (1996) Phenotypic markers for selection of nematode-resistant sheep. International Journal for Parasitology **26**:899-911

Douch PG, Harrison GB, Elliott DC, Buchanan LL, Greer KS (1986) Relationship of gastrointestinal histology and mucus antiparasite activity with the development of resistance to trichostrongyle infections in sheep. Veterinary Parasitology **20**:315-331

Douch PGC, Green RS, Morris CA, Bisset SA, Vlassoff A, Baker RL, Watson TG, Hurford AP, Wheeler M (1995b) Genetic and phenotypic relationships among anti-*Trichostrongylus colubriformis* antibody level, faecal egg count and body weight traits in grazing Romney sheep. Livestock Production Science **41**:121-132

Douch PGC, Outteridge PM (1989) The relationship betwen ovine lymphocyte antigens and parasitological and production parameters in Romney sheep. International Journal for Parasitology **19**:35-41

Dubucquoi S, Desreumaux P, Janin A, Klein O, Goldman M, Tavernier J, Capron A, Capron M (1994) Interleukin 5 synthesis by eosinophils: association with granules and immunoglobulin-dependent secretion. Journal of Experimental Medicine **179**:703-708

Eady SJ, Woolaston RR, Barger IA (2002) Comparison of genetic and nongenetic strategies for control of gastrointestinal nematodes of sheep. Livestock Production Science **81**:11-23

Eady SJ, Woolaston RR, Lewer RP, Raadsma HW, Swan AA, Ponzoni RW (1998) Resistance to nematode parasites in Merino sheep: correlation with production traits. Australian Journal of Agricultural Science **49**:1201-1211

Elard L, Sauve C, Humbert JF (1998) Fitness of benzimidazole-resistant and -susceptible worms of *Teladorsagia circumcincta*, a nematode parasite of small ruminants. Parasitology **117**:571-578

Emery DL (1996) Vaccination against worm parasites of animals. Veterinary Parasitology **64**:31-45

Emery DL, McClure SJ, Wagland BM (1993) Production of vaccines against gastrointestinal nematodes of livestock. Immunology & Cell Biology **71**:463-472

Faedo M, Barnes EH, Dobson RJ, Waller PJ (1998) The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep: pasture plot study with *Duddingtonia flagrans*. Veterinary Parasitology **76**:129-135

Faedo M, Larsen M, Waller PJ (1997) The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep: comparison between Australian isolates of Arthrobotrys spp. and *Duddingtonia flagrans*. Veterinary Parasitology **72**:149-155

FAO-STAT. sheep population. FAO-STAT Agriculture . 2003.

Fernandez S, Sarkunas M, Roepstorff A (2001) Survival of infective *Ostertagia ostertagi* larvae on pasture plots under different simulated grazing conditions. Veterinary Parasitology **96**:291-299

Forsthuber T, Yip HC, Lehmann PV (1996) Induction of TH1 and TH2 immunity in neonatal mice. Science **271**:1728-1730

Fox MT, Gerrelli D, Pitt SR, Jacobs DE, Gill M, Gale DL (1989a) *Ostertagia ostertagi* infection in the calf: effects of a trickle challenge on appetite, digestibility, rate of passage of digesta and liveweight gain. Research in Veterinary Science **47**:294-298

Fox MT, Gerrelli D, Shivalkar P, Jacobs DE (1989b) Effect of omeprazole treatment on feed intake and blood gastrin and pepsinogen levels in the calf. Research in Veterinary Science **46**:280-282

Gettinby G, Soutar A, Armour J, Evans P (1989) Anthelmintic resistance and the control of ovine ostertagiasis: A drug action model for genetic selection. International Journal for Parasitology **19**:369-376

Gibson TE, Gibson TE (1973) Recent advances in the epidemiology and control of parasitic gastroenteritis in sheep. [Review] [42 refs]. Veterinary Record **92**:469-473

Gill HS (1991) Genetic control of acquired resistance to haemonchosis in Merino lambs. Parasite Immunology **13**:617-628

Gill HS, Gray GD, Watson DL, Husband AJ (1993) Isotype-specific antibody responses to *Haemonchus contortus* in genetically resistant sheep. Parasite Immunology **15**:61-67

Gill HS, Husband AJ, Watson DL, Gray GD (1994) Antibody-containing cells in the abomasal mucosa of sheep with genetic resistance to *Haemonchus contortus*. Research in Veterinary Science **56**:41-47

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

Githigia SM, Thamsborg SM, Larsen M (2001) Effectiveness of grazing management in controlling gastrointestinal nematodes in weaner lambs on pasture in Denmark. Veterinary Parasitology **99**:15-27

Githigia SM, Thamsborg SM, Larsen M, Kyvsgaard NC, Nansen P (1997) The preventive effect of the fungus *Duddingtonia flagrans* on trichostrongyle infections of lambs on pasture. International Journal for Parasitology **27**:931-939

Grafen A, Woolhouse MEJ (1993) Does the negative binomial distribution add up? Parasitology Today **9**:475-477

Green P, Falls K, Crooks S (1990) Cri-map version 2.4. St. Louis, MO, Washington University School of Medicine

Gregory RD, Woolhouse MEJ (1993) Quantification of parasite aggregation: a simulation study. Acta Tropica **54**:131-139

Grenfell BT, Wilson K, Isham VS, Boyd HEG, Dietz K (1995) Modelling patterns of parasite aggregation in natural populations: trichostrongylid nematode-ruminant interactions as a case study. Parasitology **111**:S135-S151

Gulland FM (1992) The role of nematode parasites in Soay sheep (Ovis aries L.) mortality during a population crash. Parasitology **105**:493-503

Gulland FM, Fox M (1992) Epidemiology of nematode infections of Soay sheep (Ovis aries L.) on St Kilda. Parasitology **105 (Pt 3)**:481-492

Hein WR (1998) Immunoglobulins. In: Hand book of vertebrate immunology. Academic Press., London, UK

Henderson NG (2002) Immunity to *Teladorsagia circumcincta* infection in Scottish Blackface sheep:
An investigation into the kinetics of the immune response, antigen recognition and the MHC. PhD Thesis University of Glasgow.

Herd RP, Queen WG, Majewski GA (1992) Sex-related susceptibility of bulls to gastrointestinal parasites. Veterinary Parasitology **44**:119-125

Hilderson H, Berghen P, De Graaf DC, Claerebout E, Vercruysse J (1995) Immunisation of calves with *Ostertagia ostertagi* fourth stage larval antigens failed to protect calves from infection. International Journal for Parasitology **25**:757-760

Holmes PH (1993) Interactions between parasites and animal nutrition - the veterinary consequences. Proceedings of the Nutrition Society **52**:113-120

Hunter GC, Quenouille MH (1952) A statistical examination of the worm egg count sampling technique for sheep. Journal of Helminthology **26**:157-170

Huntley JF, Gibson S, Brown D, Smith WD, Jackson F, Miller HR (1987) Systemic release of a mast cell proteinase following nematode infections in sheep. Parasite Immunology **9**:603-614

Huntley JF, Newlands GF, Jackson F, Miller HR (1992) The influence of challenge dose, duration of immunity, or steroid treatment on mucosal mast cells and on the distribution of sheep mast cell proteinase in Haemonchus-infected sheep. Parasite Immunology **14**:429-440

Jackson F (1993) Anthelmintic resistance--the state of play. British Veterinary Journal **149**:123-138

Janssen M, Weiimann C, Gauly M, Erhardt G (2002) Association between infections with Haemonchus contortus and genetic markers on ovine chromosome 20. Proceedings of the 7th World Congress on Genetics Applied to Livestock Production

Jones WO, Emery DL, McClure SJ, Wagland BM (1994) Changes in inflammatory mediators and larval inhibitory activity in intestinal contents and mucus during primary and challenge infections of sheep with *Trichostrongylus colubriformis*.. International Journal for Parasitology **24**:519-525

Kahn LP, Knox MR, Walkden-Brown SW, Lea JM (2003) Regulation of the resistance to nematode parasites of single- and twin-bearing Merino ewes through nutrition and genetic selection. Veterinary Parasitology **114**:15-31

Kassai T, Fesus L, Hendrikx WM, Takats C, Fok E, Redl P, Takacs E, Nilsson PR, Van Leeuwen MA, Jansen J, . (1990) Is there a relationship between haemoglobin genotype and the innate resistance to experimental Haemonchus contortus infection in Merino lambs? Veterinary Parasitology **37**:61-77

Keeling MJ (2000) Simple stochastic models and their power-law type behaviour. Theoretical Population Biology **58**:21-31

Kilpatrick AM, Ives AR (2003) Species interactions can explain Taylor's power law for ecological time series. Nature **422**:65-68

Knott SA, Elsen JM, Haley CS (1996) Methods for muliple-marker mapping of quantitative trait loci in half-sib populations. Theoretical and Applied Genetics **93**:71-80

Knox DP (2000) Development of vaccines against gastrointestinal nematodes. Parasitology **120**:S43-S61

Knox DP, Redmond DL, Newlands GF, Skuce PJ, Pettit D, Smith WD (2003) The nature and prospects for gut membrane proteins as vaccine candidates for *Haemonchus contortus* and other ruminant trichostrongyloids. International Journal for Parasitology **33**:1129-1137

Knox MR, Faedo M (2001) Biological control of field infections of nematode parasites of young sheep with *Duddingtonia flagrans* and effects of spore intake on efficacy. Veterinary Parasitology **101**:155-160

Kooyman FN, Van Kooten PJ, Huntley JF, MacKellar A, Cornelissen AW, Schallig HD (1997) Production of a monoclonal antibody specific for ovine immunoglobulin E and its application to monitor serum IgE responses to *Haemonchus contortus* infection. Parasitology **114**:395-406

Koski KG, Scott ME (2001) Gastrointestinal nematodes, nutrition and immunity: breaking the negative spiral. Annual Review of Nutrition **21**:297-321

Larsen JW, Anderson N, Vizard AL, Anderson GA, Hoste H (1994) Diarrhoea in merino ewes during winter: association with trichostrongylid larvae. Australian Veterinary Journal **71**:365-372

Larsen M (2000) Prospects for controlling animal parasitic nematodes by predacious micro fungi. Parasitology **120**:S121-S131

Leathwick DM, Vlassoff A, Barlow ND (1995) A model for nematodiasis in New Zealand lambs: the effect of drenching regime and grazing management on the development of anthelmintic resitance. International Journal for Parasitology **25**:1479-1490

Littell RC, Milliken GA, Stroup WW, Wolfinger RD (1996) SAS System for Mixed Models. SAS Institute, Inc., Cary, North Carolina

Maizels RM, Bundy DAP, Selkirk ME, Smith DF, Anderson RM (1993) Immunological modulation and evasion by helminth parasites in human populations. Nature **365**:797-805

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

McEwan JC, Dodds KG, Greer GJ, Bain WE, Duncan SJ, Wheeler R, Knowler KJ, Reid PJ, Green RS, Douch PGC (1995) Genetic estimates for parasite resistance traits in sheep and their correlations with production traits. New Zealand Journal of Zoology **22**:177

Mcgillivery DJ, Yong WK, Adler B, Riffkin GG (1992) A purified stage-specific 31 kda antigen as a potential protective antigen against *ostertagia-circumcincta* infection in lambs. Vaccine **10**:607-613

McKellar QA (1993) Interactions of Ostertagia species with their bovine and ovine hosts. International Journal for Parasitology **23**:451-462

McKellar QA, Marriner SE (1987) Comparison of the anthelmintic efficacy of oxfendazole or ivermectin administered orally and ivermectin administered subcutaneously to sheep during the periparturient period. Veterinary Record **120**:383-386

Michel JF (1976) The epidemiology and control of some nematode infections of grazing animals. Advances in Parasitology355-397

Miller FM, Blair HT, Reynolds GW, Revell DK (1998) The role of cysteine in the increased parasite susceptibility of Romney sheep selected for hogget fleece weight. Proceedings of the New Zealand Society of Animal Production. **58**:153-158

Miller HR (1984) The protective mucosal response against gastrointestinal nematodes in ruminants and laboratory animals. Veterinary Immunology & Immunopathology **6**:167-259

Miller HR (1996) Prospects for the immunological control of ruminant gastrointestinal nematodes: natural immunity, can it be harnessed?. International Journal for Parasitology **26**:801-811

Miller HR, Jackson F, Newlands G, Appleyard WT (1983) Immune exclusion, a mechanism of protection against the ovine nematode *Haemonchus contortus*. Research in Veterinary Science **35**:357-363

Miller HR, Nawa Y (1979) Nippostrongylus brasiliensis: intestinal goblet-cell response in adoptively immunized rats. Exp. Parasitol. **47**:81-90

Miller JE, Baker DG, Gershwin LJ, Canalas J, Kearney MT (1996) Serum IgE levels in dairy calves: evaluation of parasite and pasture exposure as possible determinants of IgE response. Veterinary Parasitology **63**:337-343

Mitchell GB, Fitzsimons J (1983) Control of ovine gastrointestinal helminthiasis by the use of 'clean' grazing and strategic dosing in the field. Research in Veterinary Science **35**:100-105

Morris CA (1998) Responses to selection for disease resistance in sheep and cattle in New Zealand and Australia. Proceedings of the 6th World Congress on Genetics Applied to Livestock Production **27**:295-302

Morton RE, Yong WK, Riffkin GG, Bozas SE, Spithill TW, Adler B, Parsons JC (1995) Inability to reproduce protection against *Teladorsagia circumcincta* in sheep with a purified stage-specific 31 kDa antigen complex. Vaccine **13**:1482

Munn EA, Smith TS, Graham M, Greenwood CA, Tavernor AS, Coetzee G (1993) Vaccination of merino lambs against haemonchosis with membrane-associated proteins from the adult parasite. Parasitology **106**:63-66

Murray J, Smith WD (1994) Ingestion of host immunoglobulin by three non-blood-feeding nematode parasites of ruminants. Research in Veterinary Science **57**:387-389

Murray M, Miller HRP, Jarrett WFH (1968) The globule leukocyte and its derivation from the subepithelial mast cell. Laboratory Investigation **19**:222-234

Nansen P, Larsen M, Gronvold J, Wolstrup J, Zorn A, Henriksen SA (1995) Prevention of clinical trichostrongylidosis in calves by strategic feeding with the predacious fungus *Duddingtonia flagrans*. Parasitology Research **81**:371-374

Newlands GF, Skuce PJ, Knox DP, Smith SK, Smith WD (1999) Cloning and characterization of a beta-galactoside-binding protein (galectin) from the gut of the gastrointestinal nematode parasite *Haemonchus contortus*. Parasitology **119**:483-490

Newton SE, Monti JR, Greenhalgh CJ, Ashman K, Meeusen ENT (1997) cDNA cloning of galectins from third stage larvae of the parasitic nematode *Teladorsagia circumcincta*. Molecular and Biochemical Parasitology **86**:143-153

Newton SE, Munn EA (1999) The development of vaccines against gastrointestinal nematode parasites, particularly *Haemonchus contortus*. Parasitology Today **15**:116-122

Nicholas FW (1987) Veterinary Genetics. Oxford University Press, Oxford

Niezen JH, Charleston WA, Hodgson J, Miller CM, Waghorn TS, Robertson HA (1998a) Effect of plant species on the larvae of gastrointestinal nematodes which parasitise sheep. International Journal for Parasitology **28**:791-803

Niezen JH, Waghorn GC, Charleston WA (1998b) Establishment and fecundity of *Ostertagia circumcincta* and *Trichostrongylus colubriformis* in lambs fed lotus (Lotus pedunculatus) or perennial ryegrass. Veterinary Parasitology **78**:13-21

Outteridge PM, Stewart DJ, Skerman TM, Dufty JH, Egerton JR, Ferrier G, Marshall DJ (1989) A positive association between resistance to ovine footrot and particular lymphocyte antigen types. Aust. Vet. J. **66**:175-179

Parkins JJ, Holmes PH (1989) Effects of gastrointestinal helminth parasites on ruminant nutrition. Nutrition Research Reviews **2**:227-246

Parkinson J, Mitreva M, Whitton C, Thomson M, Daub J, Martin J, Schmid R, Hall N, Barrell B, Waterston RH, McCarter JP, Blaxter ML (2004) A transcriptomic analysis of the phylum Nematoda.[see comment]. Nature Genetics **36**:1259-1267

Paterson AM, Banks J (2001) Analytical approaches to measuring cospeciation of host and parasites: through a glass, darkly. International Journal for Parasitology **31**:1012-1022

Paterson S, Wilson K, Pemberton JM (1998) Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population (*Ovis aries L.*). Proceedings of the National Academy of Sciences of the United States of America **95**:3714-3719

Pena MT, Miller JE, Fontenot ME, Gillespie A, Larsen M (2002) Evaluation of *Duddingtonia flagrans* in reducing infective larvae of *Haemonchus contortus* in feces of sheep. Veterinary Parasitology **103**:259-265

Perry BD, Randolph TF (1999) Improving the assessment of the economic impact of parasitic diseases and of their control in production animals. [Review] [55 refs]. Veterinary Parasitology **84**:145-168

Perry JN (1981) Taylor's power law for dependence of variance on mean in animal populations. Applied Statistics **30**:254-263

Poulin R (1996) Helminth growth in vertebrate hosts: does host sex matter? International Journal for Parasitology **26**:1311-1315

Preston JM, Allonby EW (1979) The influence of haemoglobin phenotype on the susceptibility of sheep to *Haemonchus contortus* infection in Kenya. Research in Veterinary Science **26**:140-144

Reid JF, Armour J (1972) Seasonal fluctuations and inhibited development of gastro-intestinal nematodes of sheep. Research in Veterinary Science **13**:225-229

Reid JFS, Armour J (1975) Studies in Scottish hill sheep. I. Changes in the susceptibility of the breeding ewe to *Ostertagia* spp. Journal of Comparative Pathology **85**:163-170

Roberts JL, Swan RA (1981) Quantitative studies of ovine haemonchosis. I. Relationship between faecal egg counts and total worm counts. Veterinary Parasitology **8**:165-171

Roberts JL, Swan RA (1982) Quantitative studies of ovine haemonchosis. 2. Relationship between total worm counts of *Haemonchus contortus,* haemoglobin values and bodyweight. Veterinary Parasitology **9**:201-209

Roberts MG, Smith G, Grenfell BT (2000) Mathematical models for macroparasites of wildlife.

Roitt I, Brostoff J, Male D (2001) Immunology. Mosby, London, UK

Rothwell TL (1989) Immune expulsion of parasitic nematodes from the alimentary tract. International Journal for Parasitology **19**:139-168

Rowe JB, Nolan JV, de Chaneet G, Teleni E, Holmes PH (1988) The effect of haemonchosis and blood loss into the abomasum on digestion in sheep. British Journal of Nutrition **59**:125-139

Sangster NC (1999) Anthelmintic resistance: past, present and future. International Journal for Parasitology **29**:115-124

Sangster NC, Rickard JM, Hennessy DR, Steel JW, Collins GH (1991) Disposition of oxfendazole in goats and efficacy compared with sheep. Research in Veterinary Science **51**:258-263

Sarkunas M, Larsen M, Nansen P, Hansen JW (2000) Biological control of trichostrongylid infections in calves on pasture in Lithuania using *Duddingtonia flagrans*, a nematode-trapping fungus. Journal of Helminthology **74**:355-359

Schwaiger FW, Gostomski D, Stear MJ, Duncan JL, McKellar QA, Epplen JT, Buitkamp J (1995) An ovine *major histocompatibility complex DRB1* allele is associated with low faecal egg counts following natural predominantly *Ostertagia circumcincta* infection. International Journal for Parasitology **25**:815-822

Scudamore CL, Thornton EM, McMillan L, Newlands GF, Miller HR (1995) Release of the mucosal mast cell granule chymase, rat mast cell protease-II, during anaphylaxis is associated with the rapid development of paracellular permeability to macromolecules in rat jejunum. Journal of Experimental Medicine **182**:1871-1881

Seaton DS, Jackson F, Smith WD, Angus KW (1989) Development of immunity to incoming radiolabelled larvae in lambs continuously infected with *Ostertagia circumcincta.* Research in Veterinary Science **46**:241-246

Seaton G, Haley CS, Knott SA, Kearsey M, Visscher PM (2002) QTL Express: mapping quantitative trait loci in simple and complex pedigrees. Bioinformatics **18**:339-340

Shaw DJ, Dobson AP (1995) Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. Parasitology **111**:s111-s133

Sheldrake RF, Husband AJ, Watson DL, Cripps AW (1984) Selective transport of serum-derived IgA into mucosal secretions. Journal of Immunology **132**:363-368

Sinski E, Bairden K, Duncan JL, Eisler MC, Holmes PH, McKellar QA, Murray M, Stear MJ (1995a) Local and plasma antibody responses to the parasitic larval stages of the abomasal nematode *Ostertagia circumcincta*. Veterinary Parasitology **59**:107-118

Sinski E, Bairden K, Duncan JL, Eisler MC, Holmes PH, McKellar QA, Murray M, Stear MJ (1995b) Local and plasma antibody-responses to the parasitic larval stages of the abomasal nematode *Ostertagia circumcincta*. Veterinary Parasitology **59**:107-118

Smith G (2000) Macroparasite group report: Problems in modelling the dynamics of macroparasitic systems.

Smith WD (1993) Protection in lambs immunised with *Haemonchus contortus* gut membrane proteins. Research in Veterinary Science **54**:94-101

Smith WD (1999) Prospects for vaccines of helminth parasites of grazing ruminants. International Journal for Parasitology **29**:17-24

Smith WD, Jackson F, Jackson E, Williams J (1983) Studies on the local immune response of the lactating ewe infected with Ostertagia circumcincta. Journal of Comparative Pathology **93**:295-305

Smith WD, Jackson F, Jackson E, Williams J (1985) Age immunity to *Ostertagia circumcincta*: comparison of the local immune responses of 4 1/2- and 10-month-old lambs. Journal of Comparative Pathology **95**:235-245

Sokal R, Rohlf FJ (1995) Biometry. W. H. Freeman, New York

Soulsby EJL (1986) Helminths, Arthropods and Protozoa Of Domesticated Animals. Bailliere Tindall, London

Sreter T, Molnar V, Kassai T (1994) The distribution of nematode egg counts and larval counts in grazing sheep and their implications for parasite control. International Journal for Parasitology **24**:103-108

Stear MJ, Abuargob O, Benothman M, Bishop SC, Innocent GT, Kerr A, Mitchell S (2005a) Variation among faecal egg counts following natural nematode infection in Scottish Blackface lambs. Parasitology (In Press)

Stear MJ, Bairden K, Bishop SC, Buitkamp J, Epplen JT, Gostomski D, McKellar QA, Schwaiger FW, Wallace DS (1996a) An ovine lymphocyte antigen is associated with reduced faecal egg counts in four-month-old lambs following natural, predominantly *Ostertagia circumcincta* infection. International Journal for Parasitology **26**:423-428

Stear MJ, Bairden K, Bishop SC, Duncan JL, Karimi SK, McKellar QA, Murray M (1995a) Different patterns of faecal egg output following infection of Scottish Blackface lambs with *Ostertagia circumcincta.* Veterinary Parasitology **59**:29-38

Stear MJ, Bairden K, Bishop SC, Gettinby G, McKellar QA, Park M, Strain SAJ, Wallace DS (1998) The processes influencing the distribution of parasitic nematodes among naturally infected lambs. Parasitology **117**:165-171

Stear MJ, Bairden K, Duncan JL, Eckersall PD, Fishwick G, Graham PA, Holmes PH, McKellar QA, Mitchell S, Murray M, Parkins JJ, Wallace DS (2000) The influence of relative resistance and urea-supplementation on deliberate infection with *Teladorsagia circumcincta* during winter. Veterinary Parasitology **94**:45-54

Stear MJ, Bairden K, Duncan JL, Gettinby G, McKellar QA, Murray M, Wallace DS (1995b) The distribution of faecal nematode egg counts in Scottish Blackface lambs following natural, predominantly *Ostertagia circumcincta* infection. Parasitology **110**:573-581

Stear MJ, Bairden K, Duncan JL, Holmes PH, McKellar QA, Park M, Strain SAJ, Murray M (1997) How hosts control worms. Nature **389**:27

Stear MJ, Bairden K, McKellar QA, Scott I, Strain SAJ (1999a) The relationship between the number and size of nematodes in the abomasum and the concentration of pepsinogen in ovine plasma. Research in Veterinary Science **67**:89-92

Stear, M. J., Baldock, F. C., Brown, S. C., Gershwin, L. J., Hetzel, D. J. S., Miller, J. E., Nicholas, F. W., Rudder, T. H., and Tierney, T. J. The genetic control of nematode infections in ruminants. Fourth world congress on genetics applied to livestock production XVI: 449-452. 1990.

Stear MJ, Bishop SC (1999) The curvilinear relationship between worm length and fecundity of *Teladorsagia circumcincta.* International Journal for Parasitology **29**:777-780

Stear MJ, Bishop SC, Doligalska M, Duncan JL, Holmes PH, Irvine J, McCririe L, McKellar QA, Sinski E, Murray M (1995c) Regulation of egg production, worm burden, worm length and worm fecundity by host responses in sheep infected with *Ostertagia circumcincta.* Parasite Immunology **17**:643-652

Stear MJ, Bishop SC, Duncan JL, McKellar QA, Murray M (1995d) The repeatability of faecal egg counts, peripheral eosinophil counts, and plasma pepsinogen concentrations during deliberate infections with *Ostertagia circumcincta.* International Journal for Parasitology **25**:375-380

Stear MJ, Bishop SC, Henderson NG, Scott I (2003) A key mechanism of pathogenesis in sheep infected with the nematode Teladorsagia circumcincta. [Review] [90 refs]. Animal Health Research Reviews **4**:45-52

Stear MJ, Bishop SC, Mallard BA, Raadsma H (2001a) The sustainability, feasibility and desirability of breeding livestock for disease resistance. [Review] [92 refs]. Research in Veterinary Science **71**:1-7

Stear MJ, Eckersall PD, Graham PA, McKellar QA, Mitchell S, Bishop SC (2001b) Fructosamine concentration and resistance to natural, predominantly Teladorsagia circumcincta infection. Parasitology **123**:211-218

Stear MJ, Eckersall PD, Graham PA, McKellar QA, Mitchell S, Bishop SC (2001c) Fructosamine concentration and resistance to natural, predominantly *Teladorsagia circumcincta* infection. Parasitology **123**:211-218

Stear MJ, Henderson NG, Kerr A, McKellar QA, Mitchell S, Seeley C, Bishop SC (2002a) Eosinophilia as a marker of resistance to *Teladorsagia circumcincta* in Scottish Blackface lambs. Parasitology **124**:553-560

Stear MJ, Henderson NG, Kerr A, McKellar QA, Mitchell S, Seeley C, Bishop SC (2002b) Eosinophilia as a marker of resistance to *Teladorsagia circumcincta* in Scottish Blackface lambs. Parasitology **124**:553-560

Stear MJ, Innocent GT, Buitkamp J (2005b) The evolution and maintenance of polymorphism in the major histocompatibility complex. Veterinary Immunology and Immunopathology **In press**:

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

Stear MJ, Park M, Bishop SC (1996b) The key components of resistance to *Ostertagia circumcincta* in lambs. Parasitology Today **12**:438-441

Stear MJ, Strain SAJ, Bishop SC (1999b) How lambs control infection with *Ostertagia circumcincta*. Veterinary Immunology and Immunopathology **72**:213-218

Stear MJ, Wakelin D (1998) Genetic resistance to parasitic infection. Revue Scientifique et Technique **17**:143-153

Strain SAJ, Bishop SC, Henderson NG, Kerr A, McKellar QA, Mitchell S, Stear MJ (2002) The genetic control of IgA activity against *Teladorsagia circumcincta* and its association with parasite resistance in naturally infected sheep. Parasitology **124**:545-552

Strain SAJ, Stear MJ (1999) The recognition of molecules from fourth-stage larvae of *Ostertagia circumcincta* by IgA from infected sheep. Parasite Immunology **21**:163-168

Sutherland IA, Moen IC, Leathwick DM (2002) Increased burdens of drug-resistant nematodes due to anthelmintic treatment. Parasitology **125**:375-381

Sykes AR (1994) Parasitism and production in farm animals. Animal Production **59**:155-172

Sykes AR, Coop RL (1977) Intake and utilization of food by growing sheep with abomasal damage caused by daily dosing with *Ostertagia circumcincta* larvae. Journal of Agricultural Science **88**:671-677

Symons LE (1985) Anorexia: occurrence, pathophysiology, and possible causes in parasitic infections. Advances in Parasitology **24**:103-133

Tavernor AS, Smith TS, Langford CF, Graham M, Munn EA (1992) Immune response of Clun Forest sheep to vaccination with membrane glycoproteins from *Haemonchus contortus*. Parasite Immunology **14**:671-675

Taylor EL (1939) Technique for estimation of pasture infestation by strongyle larvae. Parasitology **31**:473-478

Taylor LR (1961) Aggregation, variance and the mean. Nature **189**:732-735

Thomas RJ, Boag B (1972) Epidemiological studies on gastro-intestinal nematode parasites of sheep. Infection patterns on clean and summer-contaminated pasture. Research in Veterinary Science **13**:61-69

Thomas RJ, Boag B (1973) Epidemiological studies on gastro-intestinal nematode parasites of sheep. The control of infection in lambs on contaminated pasture. Research in Veterinary Science **15**:238-249

Urban JF, Fayer R, Sullivan C, Goldhill J, Shea-Donohue T, Madden K, Morris SC, Katona I, Gause W, Ruff M, Mansfield LS, Finkelman FD (1996) Local TH1 and TH2 responses to parasitic infection in the intestine: regulation by IFN-gamma and IL-4. Veterinary Immunology & Immunopathology **54**:337-344

Urquhart GM, Armour J, Duncan JL, Jennings FW (1996) Veterinary Parasitology. Blackwell Science Ltd,

van Houtert MFJ, Sykes AR (1996) Implications of nutrition for the ability of ruminants to withstand gastrointestinal nematode infections. International Journal for Parasitology **26**:1151-1167

Vercruysse J, Claerebout E (2001) Treatment vs non-treatment of helminth infections in cattle: defining the threshold. Veterinary Parasitology **98**:195-214

Visscher PM, Thompson R, Haley CS (1996) Confidence intervals in QTL mapping by bootstrapping. Genetics **143**:1013-1020

Wakelin D (1996) Immunity to parasites; How Parasitic Infection Are Controlled. Cambridge University Press, Cambridge

Waller PJ (1994) The development of anthelmintic resistance in ruminant livestock. Acta Tropica **56**:233-243

Waller PJ (2003) Global perspectives on nematode parasite control in ruminant livestock: the need to adopt alternatives to chemotherapy, with emphasis on biological control. Animal Health Research Reviews **4**:35-43

Waller PJ, Faedo M (1996) The prospects for biological control of the free-living stages of nematode parasites of livestock. International Journal for Parasitology **26**:915-925

Waller PJ, Thomas RJ (1978) Nematode parasitism in sheep in North-East England: The epidemiology of *Ostertagia* species. International Journal for Parasitology **8**:275-283

Watson DL, Colditz IG, Andrew M, Gill HS, Altmann KG (1994) Age-dependent immune response in Merino sheep. Research in Veterinary Science **57**:152-158

Watson DL, Gill HS (1991) Post natal ontogeny of immunological responsiveness in Merino sheep. Research in Veterinary Science **51**:88-93

Wedrychowicz H, Bairden K, Dunlop EM, Holmes PH, Tait A (1995) Immune response of lambs to vaccination with *Ostertagia circumcincta* surface antigens eliciting bile antibody responses. International Journal for Parasitology **25**:1111-1121

Wedrychowicz H, Bairden K, Tait A, Holmes PH (1992) Immune responses of sheep to surface antigens of infective larvae of *Ostertagia circumcincta*. Parasite Immunology **14**:249-266

Wells PD (1963) Mucine-secreting cells in rats infected with Nippostrongylus brasiliensis. Experimental Parasitology **14**:15-28

Windon RG, Dineen JK (1981) The effect of selection of both sire and dam on the response of F1 generation lambs to vaccination with irradiated *Trichostrongylus colubriformis* larvae. International Journal for Parasitology **11**:11-18

Woolaston RR (1992) Selection of Merino sheep for increased and decreased resistance to *Haemonchus contortus:* peri-parturient effects on faecal egg counts. International Journal for Parasitology **22**:947-953

Woolaston RR, Baker RL (1996) Prospects of breeding small ruminants for resistance to internal parasites. International Journal for Parasitology **26**:845-855

Woolaston RR, Windon RG (2001) Selection of sheep for response to *Trichostrongylus colubriformis* larvae: genetic parameters. Animal Science **73**:41-48

Zinkernagel RM, Doherty PC (1979) MHC-restricted cytotoxic T cells: studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction-specificity, function, and responsiveness. Adv. Immunol. **27**:51-177

Zuk M, McKean KA (1996) Sex differences in parasite infections: patterns and processes. International Journal for Parasitology **26**:1009-1023

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