COMPARATIVE STUDIES ON BREED RESISTANCE TO OVINE HAEMONCHOSIS IN KENYA

By

John Mutua Mugambi (B.V.M., M. Sc.)

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

Department of Veterinary Parasitology

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

DECLARATION

I declare that the work described in this thesis is my original work, any collaboration and assistance having been duly acknowledged.

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DEDICATION

Posthumously To My Late Wife KARIMI

To My Parents

To My Two Children, NKIROTE and MUTETHIA

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ABSTRACT

Control of ovine haemonchosis, which is an endemic tropical and sub-tropical helminth disease of considerable economic importance, relies on anthelmintics and grazing management. Practical problems with grazing management, the occurrence of anthelmintic resistance and the cost of anthelmintics have directed attention to possible alternative control strategies. One such strategy is the use of host genetic resistance, a phenomenon known to exist for many years but which has received close attention only within the last twenty years. Within that period the Red Maasai sheep breed in Kenya was shown to be remarkably more resistant to haemonchosis than a number of other breeds. The studies described in this thesis were initiated to confirm this resistance with a view to exploring the prospect of using host resistance to control the disease in the country. In the first experimental chapter of the thesis, 15 wethers each of the Red Maasai, Romney Marsh, Dorper and Blackhead Somali breeds were left to graze contaminated pasture for one year, each breed grazing its own paddock. Egg count results confirmed earlier field studies that the Red Maasai was more resistant to haemonchosis than the Dorper and the Blackhead Somali. The results also showed that the breed was more resistant than the Romney Marsh. While the mean weekly faecal nematode egg count over 52 weeks did not exceed 2000 epg in the Red Maasai, maximum counts of 14,000, 5200 and 7200 epg were recorded for the Romneys, Dorpers and Blackhead Somali respectively. In addition no Red Maasai died, while nine Romneys, two Dorpers and two Blackhead Somali sheep died of haemonchosis. By plotting successive cumulative mean egg counts for each breed, it was shown that the potential for infection in young lambs would be much lower in pastures grazed by the Red Maasai alone compared to pastures grazed by any of the other breeds. The observed packed cell volume (PCV) changes appeared to be related to the susceptibility of the breed. An interesting feature of this study was the similar pattern of egg counts and PCVs observed with the Dorper and the Blackhead

Somali sheep and probably reflected their genetic relationship. Serum anti-*Haemonchus contortus* L_3 IgGs were clearly delineated between indigenous and 'exotic' breeds with the former having higher values. The similarity in the pattern and level of faecal egg counts and PCVs observed with the Dorpers and the Blackhead Somali sheep was not observed with the IgG responses. Apart from the high mortality rate, the Romneys also showed substantial weight loss. Although the other breeds showed overall weight gains these were probably lower than they would have achieved with appropriate anthelmintic treatment.

Following artificial infection of the surviving wethers with a single dose of 10,000 *H. contortus* L_3, the differences in faecal egg output and PCVs were maintained. However, no valid comparison could be made between the Romneys and the other breeds because only three animals were available for infection. At necropsy eight weeks after infection, no significant difference in worm counts was observed between the three breeds implying that worm fecundity might be a target of the resistance mechanism of the Red Maasai. The significantly higher PCVs of the Red Maasai compared with the Dorper and the Blackhead Somali may be a reflection of differences in haemopoietic responses to infection. Alternatively, or in addition, there may have been a greater worm establishment but a greater subsequent loss of worms in the Dorpers and the Blackhead Somali. The order of increasing susceptibility was Red Maasai, Blackhead Somali, Dorper and Romney Marsh.

In the second experiment, maiden Dorper and Red Maasai ewes were infected with a single dose of 5000 *H. contortus* L3 and after nine weeks of sampling, they were treated and turned out to graze together on the same contaminated pasture. Sampling continued outdoors for 14 months. On the basis of faecal egg counts, the Red Maasai ewes were significantly more resistant than the Dorper ewes after both the artificial and natural infections. No significant breed by time interactions occurred implying that the differences were consistent throughout the study. Although the ewes were not necropsied for total worm counts, it

appeared that the differences were likely to be due to an effect on worm fecundity rather than on the established numbers of worms since no significant differences in PCVs occurred during both periods of the study. Also from the limited observations made during the peri-parturient period it appeared that the faecal egg output of the Dorpers remained at twice that of the Red Maasai ewes. The significant breed by time changes observed with eosinophil and serum anti-worm IgG responses indicated that breed differences occurred at certain times and these were usually in favour of the Red Maasai but overall there were no significant differences. It is possible that the level of artificial infection used in this age of ewes was not sufficient to produce appreciable differences. In addition, the fact that the ewes grazed together may have offered the Dorper ewes an advantage of a less contaminated pasture than that grazed by Dorper wethers alone, in the four breed study. It is also the case that mature female sheep are usually more resistant to *Haemonchus* infection than males.

In the third experiment two groups of 17 six month old Dorper and Red Maasai male lambs were repeatedly infected with 1000 *H. contortus* L_3 at 1-2 week intervals for the first 10 weeks of the experiment and sampled for a total of 18 weeks. A further two groups of uninfected controls were also sampled and the four groups were housed separately. There were clear differences in faecal egg counts, PCVs, total serum proteins and eosinophil counts between the infected groups with the Red Maasai being more resistant. Also, five Dorpers died of haemonchosis compared with only one Red Maasai. No differences in PCVs, eosinophil counts and total serum proteins were observed between the two control groups indicating that the differences seen between the infected groups reflected differences in breed response to infection. Interestingly weight gains were not depressed in the infected groups and this was probably due to their increased feed intake of the high protein concentrate and lucerne hay diet.

In the fourth experiment, 20 Dorper and 20 Red Maasai male lambs were given three successive infections of 5000 *H. contortus* L_3 at 8-9 week intervals

from six months of age. The lambs were housed together and supplemented with lucerne hay and a high protein concentrate. No breed differences were observed in faecal egg counts, PCVs, eosinophil counts, serum anti-*H. contortus* IgG adult or L_3 antigens or in worm counts at necropsy. In this experiment it appeared that when the Dorper is well fed it will show a similar level of resistance to the Red Maasai in situations of single large infections or of several large infections at intervals of several months since in the third experiment the animals were also well-fed yet there were clear differences between the breeds. The results of these four experiments suggest that the Red Maasai responds better to continuous or low level trickle infections than to single large infections, since the breed showed a higher level of resistance under natural or experimental trickle infections. They also suggest that with trickle infections the breed might be able to direct its resistance mechanism against both worm establishment/expulsion and fecundity and this may explain why there were no deaths during the natural exposure period of the first experiment. The eosinophil responses seen in the fourth experiment appeared to be anamnestic in nature. However the eosinophil response to the third infection was not entirely due to the infection since control lambs also showed increased counts at this time. The reasons for these increases are not known. The minimal differences in urea levels observed between control and infected lambs in this study indicated that with an adequate protein diet, differences between control and infected animals are not clearly defined.

In the fifth experiment, Dorper and Red Maasai lambs were divided into two groups. One group was infected at four months of age while the other was infected at six months. The lambs were housed and fed a diet similar to that used in the preceding experiment. The aim was to find out whether the two breeds would show any age related differences in their susceptibility to *H. contortus.* The lambs were infected with 5000 L_3 at 8-9 week intervals and the results contrasted sharply with the findings of the first three experiments in that the Dorpers appeared to be more resistant than the Red Maasai. In this experiment

the Dorpers had significantly lower faecal egg counts and higher PCVs and eosinophil counts than the Red Maasai and the Red Maasai also had a substantially higher mortality rate. The reasons for this apparent reversal in the responses of the two breeds is not known but it is possible the Red Maasai may have been receiving inadequate feed. The interpretation of the results was also complicated by the fact that prior to the experimental infections the Dorpers had inadvertently acquired substantial extraneous infection which necessitated treatment. This may have primed the Dorpers and thus modulated their expected responses. However, the fact that the Dorper was still apparently superior when infected at six months of age, that is 11 weeks after treatment does not lend support to the suggestion that extraneous infection was a cause of the reversal of the responses. No breed differences in worm counts were evident in those animals which survived to the end of the experiment. Dorper males were more susceptible than females while Red Maasai males and females showed similar susceptibilities. This probably reflected the earlier maturity attained by Dorpers. There were significant breed differences in worm lengths in lambs first infected at four months, with the Red Maasai having longer worms, but not in those infected at six months despite similar worm burdens. It thus appeared as if there was a host age effect on worm length which reduced breed differences with time. No breed differences in worm fecundity were observed in worms recovered from lambs infected at six months of age.

Overall, the results showed that the Red Maasai responded better than the Dorper under conditions of continuous or low level trickle infections as occurs in the field. This suggests that despite the anomalous situation recorded in the last experiment, the Red Maasai breed should still be superior to the Dorper in the field. However, the real differences between the two breeds were not marked possibly because the Dorper possesses some genetic background from the Blackhead Somali which is itself fairly resistant to haemonchosis. Furthermore, the similarity in faecal egg counts, PCVs and worm burdens seen between the

Dorper and the Blackhead Somali in the first experiment may indicate that since its development, the Dorper has also had some selection for resistance against haemonchosis.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 GASTROINTESTINAL PARASITISM IN SHEEP

1.1.1 General

Parasitism is one of three animal associations, the others being commensalism and symbiosis. A parasite is a smaller organism that lives in or on a larger organism called the host and is dependent on that host for survival. The host's inputs in supporting its parasites may be negligible or so substantial that they are unsustainable. This will depend on the number of parasites, the size and type of injury caused and the clinical, nutritional, age and physiological (e.g. pregnancy, lactation) status of the host.

The biological environment for the parasites within the hosts consists of several habitats located mainly in the gastrointestinal and respiratory tracts.

1.1.2 Economic impact

The total cost of parasitism is difficult to assess. While data on the costs of treatment are relatively easy to obtain, the size and value of production losses are more difficult to determine. In an extensive study in Australia in 1984-85, the average costs of parasites to the sheep industry per farm was estimated at $7460 annually, $4695 of which was due to internal parasites (Beck *et al.*, 1985). Production losses (wool loss, meat loss and deaths) accounted for 82.7% of the $7460 loss, while prevention and treatment operations accounted for the rest. Because the level of parasite infestation is largely related to climatic conditions, considerable variability in costs were found from year to year and average annual losses accordingly varied from $3300 to $6200. Also in Australia, Gray (1987) estimated that one out of every seven bales of wool perish at the point of production because of worms. In the tropics, haemonchosis is probably the greatest single constraint to sheep production (Allonby and Urquhart, 1975) but

losses have not been objectively quantified.

From the literature the main ways in which parasites, in particular gastrointestinal nematodes, can reduce returns to the farmer are:

(a) mortality, which includes the capital costs of replacement;

(b) quantitative and qualitative reductions in liveweight gain, wool growth and reproductive efficiency;

(c) increased costs of production through the requirement for anthelmintic treatments and the labour involved;

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

Results from several controlled studies indicate that production effects, that is, weight loss, decline in milk production, wool and carcass quality are associated with subclinical more than with clinical infections (Nansen, 1986).

1.1.3 Control methods

Use of anthelmintics

Anthelmintics are most often used as the primary means of parasite control (Prichard, 1990). However, for better results a measure of complementary grazing management is often included. The use of anthelmintics dates back to the end of the last century. Copper sulphate appears to be the first compound to have been used against the gastrointestinal parasites of sheep by Hutcheon (1891, cited by Gibson, 1975). It remained in common usage until 1940 when it was superseded by phenothiazine (Roberts, 1939). Veglia (1919) tested a mixture of copper sulphate and sodium arsenite against haemonchosis while a copper sulphate- nicotine sulphate mixture (the CuNic solution of Curtice) was used in sheep (Hall, 1930). Some other early compounds used as anthelmintics included carbon tetrachloride (Hall and Shillinger, 1925), tetrachloroethylene (Schlingman, 1926), piperazine (Gordon, 1955), the organophosphorus compounds such as trichlorphon (Gordon, 1958) and methyridine (Broome and

Greenhalgh, 1961).

Since the early 1960's more efficacious compounds have been discovered and developed which have the advantage of being broad spectrum and relatively non-toxic. These are used in the control of parasitic gastro-enteritis (P.G.E.) of cattle and sheep which is caused by mixed infections with several nematode species (Gibson, 1975). For the nematode parasites of livestock there are three major families of anthelmintics, i.e. the levamisole and morantel group, the benzimidazoles and the avermectins. These anthelmintics are effective against most gastrointestinal nematodes, lungworms and some tissue nematodes and in the case of benzimidazoles, some other helminths like tapeworms and liver flukes (Prichard, 1990). Moxidectin which is structurally similar to the avermectins since it is a second generation semi-synthetic macrocyclic lactone (Williams *et al.*, 1992; Hayes, 1994) is a new addition to the list.

Urquhart *et al.* (1987) discuss chemoprophylaxis of P.G.E. of sheep in temperate regions on the basis of whether farms consist mainly of permanent pasture or whether they have alternative grazing. In the former case they recommended treatment of ewes one month prior to lambing. This treatment is aimed at removing existing worms burdens, including arrested larvae, and thus eliminating the peri-parturient rise in faecal egg counts, which is an important source of infection to the new lamb crop (Salisbury and Arundel, 1970). It was also considered that this treatment would improve the body condition of ewes on extensive grazing at a time when nutritional status is frequently low. A further treatment of the ewes four to six weeks post-lambing is recommended to take care of infection derived from overwintered larvae; young adults and rams should be included in the treatment to reduce their contribution to pasture contamination. This latter treatment is particularly useful in countries like Spain where overwintered larvae give rise to substantial infection of ewes and lambs (Uriarte and Valderrabano, 1989). In the case of lambs, treatment at weaning is recommended, with subsequent movement to safe pastures where possible.

Where no safe pastures are available, one or two additional treatments would be required till late summer/autumn or marketing. In farms with alternate grazing, fewer anthelmintic treatments are required and methods of control are described below, under combined chemoprophylaxis and grazing management.

Grazing management methods

One method uses two or more different host species grazing pasture alternately, the changeover taking place when the pastures have become helminthologically safe for the alternate host. The cattle-sheep alternative is used most commonly because the important nematodes of these animals are relatively host specific so that pasture contaminated by sheep may be considered clean for cattle and vice versa (Thomas, 1982). Thus the effectiveness of this system obviously depends on the degree of cross-transmission of parasites between the different hosts (Morley and Donald, 1980). The interval between changeovers should not be so long that one host is infected by its own parasites. In practice control is best achieved by exchanging in spring pastures grazed by sheep or beef cattle over the previous year, preferably combined with an anthelmintic treatment at the time of exchange (Urquhart *et al.*, 1987).

Another management method is rotational grazing. In this case pastures are rested long enough for any residual contamination to decline to negligible levels before susceptible livestock are introduced. If not grazed, the forage can be harvested for hay or silage. Alternatively a fodder or grain crop may be grown on the area and livestock grazed directly on the crop or on the residues after harvest.

Two other systems which have been described for farms with alternate grazing but due to the costs of fencing and labour are little used nowadays, are strip and creep grazing. Strip grazing involved confining sheep along narrow strips across the field with fences which were moved every few days while in creep grazing, ewes were confined but lambs could creep out and graze ahead of the dams (Urquhart *et al.*, 1987).

Combined chemoprophylaxis and grazing management strategies

Grazing management schemes usually incorporate a limited number of anthelmintic treatments. For sheep there is the so called 'clean grazing' system which entails grazing ewes and lambs on pasture which has not carried lambs or young sheep during the last 12 months (Mitchell and Fitzsimons, 1983). This regime involves anthelmintic treatment of ewes immediately before the ewes and their lambs are introduced to clean grazing thus reducing the contribution of peri-parturient rise in ewe faecal egg output to subsequent pasture contamination. In this way any overwintered larvae will have died and the relatively clean animals are therefore being turned out onto a relatively clean pasture. Boag and Thomas (1973) found that a single treatment of either the ewes or the lambs were equally effective in reducing infection in the lambs when associated with a change of pasture at weaning. Variations based on these principles have been referred to as 'preventive' and 'evasive' strategies (Michel 1976). In a preventive strategy pasture contamination by the ewe in the early part of the grazing season is suppressed by regular treatments or the ewe is dosed before moving it on to clean pasture. With the evasive strategy, exposure to the build up of infection on the pasture in the second half of the grazing season is avoided by dosing the lambs and moving them on to 'clean aftermath' or to an area with fodder.

Descriptions of control strategies in the tropics are scanty and there is a need to address the issue (Fabiyi, 1986). Some studies (Irfan 1967) indicated that with haemonchosis, treatments in intensive husbandry systems had to be at rather short intervals of four weeks if mortalities were to be avoided and satisfactory weight gains achieved. However, since infective larvae can survive on pasture for short periods of only three to seven weeks in some tropical areas (Okon and Enyenihi, 1977; Barger *et al.*, 1994), 'spelling' of pastures for short periods may be effective in controlling infection (Fabiyi, 1986). This has been demonstrated with goats and the traditional method of tethering and movement of stock to a

new grazing area every one to two days could be an effective low-cost control measure (Barger *et al.*, 1994). However, 'spelling' is impractical in many tropical areas where farmers often own only very small pieces of land. Epidemiological studies in the 'derived' savannah regions, such as those found in Nigeria, have shown that pastures that are exclusively grazed by sheep and/or goats are free of infective larvae during the dry season which runs from December to April (Chiejina *et al.*, 1989). The only possible source of new infection on such pastures is fresh contamination from carrier animals at the start of the wet season. A single treatment of all the animals during the dry season or just prior to the onset of rains should therefore reduce pasture contamination substantially and thus prevent significant infections in the animals during the wet season, the period during which disease outbreaks generally occur (Chiejina, 1987). In the Highlands of East Africa, Dinnik and Dinnik (1961) recommended that anthelmintic treatment should be given before the rainy warm season to avoid pasture contamination by infected animals. To remove developing worm burdens before they produce many eggs and thus further contaminate the pastures during the rainy season, treatment should be repeated at intervals of about three weeks until the weather turns cold or dry. It was also recommended that these treatments should be combined, where possible, with the movement of animals to fresh pasture every three weeks to further reduce chances of re-infection. A final treatment three weeks after the end of the dangerous season was considered desirable to prevent any carry-over of infection. There is little doubt that such frequent treatments may result in the development of anthelmintic-resistant nematode strains especially of *Haemonchus*. An earlier study (Dinnik and Dinnik, 1958) indicated that the survival of *H. contortus* L_3 in pasture in the Kenya Highlands may be diminished during certain times of the year depending on air temperatures and moisture. This suggested that for some climatic areas it is possible, on the basis of meteorological information, to define periods of high risk when chemoprophylaxis would be necessary.

1.1.4 Constraints of the control methods

Anthelmintics

The use of anthelmintics is expensive not only in terms of material but also in the labour and time involved in gathering, handling and treating livestock regularly. In addition there has been an increasing problem of anthelmintic resistance. Anthelmintic resistance emerges when individual parasites are able, due to biological diversity, to tolerate the effects of a given dose of anthelmintic and continue to reproduce. Thus over a number of generations, the process of selection will allow the resistant parasites to dominate (Prichard, 1990). Selection pressure also increases dramatically if treatment intervals approach the pre-patent period of parasites. Most reports of anthelmintic resistance come from regions where the highly pathogenic abomasal parasite, *H. contortus* is endemic and frequent treatment was probably responsible for the early appearance and subsequent high levels of resistance in Australia (Waller, 1987). A resistant strain of *H. contortus* was first reported in 1962 in a flock of sheep where phenothiazine had been used for 19 years (Levine and Garrigus, 1962). A few years later, a strain resistant to thiabendazole was reported (Conway, 1964), only three years after the introduction of the anthelmintic. Since then there have been numerous reports of benzimidazole resistant nematodes from various countries including Kenya (Maingi, 1991) and there are detailed reviews on the subject (Prichard 1990).

Resistance to levamisole and morantel appeared to evolve more slowly. However, there are several reports of resistance to these drugs in *H. contortus, Trichostrongylus colubriformis* and *Ostertagia circumcincta* in sheep and goats (Sangster *et al.*, 1979; Prichard *et al.*, 1980). More recently, resistance against ivermectin has emerged. For instance a *H. contortus* resistant strain has been observed in sheep in South Africa (van Wyk and Malan, 1988) and Brazil (Echevarria and Trindade, 1989), and in goats in U.S.A. (Craig and Miller,

1990).

To reduce the labour costs involved in repeated treatments long acting devices or boluses have been developed. These are now available for sheep, for example an albendazole bolus is used to abolish the periparturient rise in faecal egg counts. Although more convenient, long acting devices are more expensive in terms of product costs compared to conventional formulations (Urquhart, 1988).

In addition to constraints of cost and anthelmintic resistance there is some evidence from cattle that animals reared under highly effective anthelmintic prophylaxis regimes during their first year of life may subsequently fail to develop a significant degree of acquired immunity (Urquhart *et al.*, 1987). This problem is likely to be more serious with slow release boluses and some studies indicate that these devices partially impair immunity development in treated calves. Thus heifers prophylactically treated with oxfendazole pulse release intraruminal devices (OPRB) to protect them against natural *Dictyocaulus viviparus* or *Ostertagia ostertagi* infections showed similar clinical responses to untreated heifers following challenge, but the larger faecal larval/egg output of the OPRB groups suggested greater worm establishment in these groups (Jacobs *et al.*, 1987). Similarly, calves treated with a morantel sustained release trilaminate bolus (MSRT) and then trickle infected with *Cooperia oncophora* and *O. ostertagi* had higher worm burdens than untreated infected control calves (Vercruysse *et al.*, 1992).

Grazing management

Any 'dose and move' system is based on the typical annual pattern of larval availability on pasture characterised by an early season overwintered population and a second wave from progeny of the overwintered population or spring rise in faecal egg output. Some deviations from this pattern may occur making the method less effective in some years (Nansen, 1986). There is another possible drawback in that any new generation of worms after the move to clean pasture

will consist of progeny which have survived treatment and thus may select for anthelmintic resistance.

Clean grazing systems generally require a reduction in grazing pressure and this reduces the potential outputs obtainable from an intensive system (Uriarte and Valderrabano, 1989). Also, there may not be enough land to allow for any resting of pastures.

The leader-follower method as in creep grazing assumes that the follower is sufficiently immune not to be infected to any great extent and this may not always be true.

In the case of alternate grazing of sheep and cattle, *Trichostrongylus axei* and *Nematodirus battus* can be cross-transmitted at a substantial level. The cross-transmission could also be unilateral. Thus while mixed grazing was effective in reducing the number of worm species normally found in sheep, the sheep simultaneously showed an increase in the number of those species normally found in cattle (Arundel and Hamilton, 1975). Also in *Haemonchus*- endemic areas it is unlikely that alternate grazing of young sheep and young cattle could be relied upon to aid in the control of this parasite in either host since sheep are sufficiently susceptible to *Haemonchus placei* to both contaminate pastures and to suffer from the effects of infection (Morley and Donald, 1980).

The quality of aftermath grazing to which lambs may be moved will vary depending on the kind of forage operation (hay or silage) and on the stage of cutting. The quantity of the aftermath may also be insufficient to provide safe grazing to a lamb crop unless fodder conservation is a major part of the farming enterprise. In addition the area required to provide fodder for the susceptible animals may be more than can economically be devoted to such a purpose.

A general constraint applicable to management is its expense. It involves new skills or concepts, costly modifications and additional resources to the extent that anthelmintics are in many occasions cheaper. The effects of management on helminth control and vice versa also seem insufficiently understood not only by

farmers but by professional advisers (Morley and Donald, 1980) and there is the additional problem of disseminating the information and demonstrating its practicability (Thomas, 1982).

1.1.5 The future of roundworm control

Waller (1987) has given some options for the control of roundworms in the future:

Chemical options

New anthelmintics- Although there appears to have been a steady stream of new anthelmintics onto the market in recent years, the majority were additional compounds in existing drug classes, notably the benzimidazole class. As far as resistance is concerned this is of little value because side-resistance within a drug class often develops rapidly. It is unlikely that new highly effective compounds will be developed in the near future since discovery through to marketing takes a long time and is very expensive. Furthermore, countries with high levels of resistance account for only a small percentage of total world sales of anthelmintics and investments by pharmaceutical companies into solving a seemingly small regional problem may not be worthwhile.

Modified anthelmintics- This aims at extending the life of existing classes of compounds by manipulating their pharmacokinetic behaviour. For instance synergistic increases in efficacy against benzimidazole resistant *H. contortus* was shown by combining mebendazole and levamisole (Bennet *et al.*, 1980).

New formulations- Incorporation of anthelmintics into sustained or controlled release devices extends the time that the drug is in contact with the parasites. This may overcome resistance problems that have evolved to single anthelmintic administration where the drug persists for only a short time within the host. Such devices are, however, very costly.

Alternative chemicals- Some compounds known as growth regulators have been

extensively evaluated to determine their usefulness as alternatives to insecticides for the control of arthropod parasites. Such compounds include growth or moulting hormones, their analogues or antagonists. Some of these have shown inhibitory activity on the development of the free-living stages of nematodes (Waller, 1987). For example, the insect growth regulator, triflumuron exhibits strong larvicidal effects against the free-living stages of *T. colubriformis* and could be a possible alternative or adjunct to conventional anthelmintic therapy (Waller and Lacey, 1986).

Better use of existing drugs- Since currently available anthelmintics will dominate control of parasitic helminths in the foreseeable future, there is an urgent need to prolong their usefulness. This can be achieved in part by designing control programmes which include fewer anthelmintic treatments. The success of two roundworm control programmes in Australia, called 'Worm Kill' and 'Drenchplan' (Waller, 1987) should encourage research in this direction.

Non-chemical options: *immunological/genetic methods*

At present, many activities are directed towards the production of helminth vaccines or the breeding of resistant hosts.

Vaccines- The precedent and drive for this approach emanated from the development of an effective and commercially viable vaccine against the bovine lung-worm *D. viviparus* (Jarrett *et al.*, 1958). However, apart from vaccines against the ovine lung worm and canine hookworm, the use of either attenuated or 'dead' vaccines against helminth parasite infections has proved disappointing (Waller, 1987). Furthermore young animals which are usually the most susceptible are often less immunologically responsive than adults and even when acquired, immunity to parasites is often short lived and not as solid as that which develops against bacteria or viruses.

Selection for resistant hosts- A variation of the immunological approach is the

improvement of overall herd or flock resistance by selection for increased natural genetically determined resistance to parasitic infection (Templeton *et al.*, 1988). Although results suggest heritability of resistance to parasites is moderately high at about 0.3 (Piper, 1987) and therefore selection for resistance should be reasonably effective (Nicholas, 1993), progress in selection will be slow due to the long generation interval of the host (Waller, 1987). Another reason for slow progress is the emphasis on selection of individuals rather than breeds particularly in Australia where Merino wool production is a specialised industry (Urquhart, 1988). Progress of selection will also be influenced by the number of genes involved; if a major resistance gene is involved selection progress would be more rapid (Albers and Gray, 1986; Kassai and Sreter, 1992).

Non-chemical methods: biological control

Use of sterile hybrids- Matings between *H. contortus* and *H. placei* produced sterile hybrids (Le Jambre, 1979) and this has been proposed as a method of control similar to that used by entomologists to control certain insects (Le Jambre, 1984). It is, however, beset with several constraints such as practicability and pre-mating barriers. These barriers prevent the two species from interbreeding naturally under field conditions. An extension of these barriers to the resulting hybrids obviously means that the hybrids would not effectively compete for mates among the wild population.

Use of fungi- Fungi which predate on or parasitise nematodes are common in decaying materials in the soil. The nematocidal activity of the ubiquitous predacious hyphomycetes has been known for almost a century and this activity is easily observed in the laboratory (Nansen *et al.*, 1988). *Arthrobotrys oligospora*, the most common species, captures free-living nematodes with hyphal nets formed when the fungus makes contact with larvae. The fungus penetrates the entrapped larvae and trophic hyphae grow inside, followed by release of a nematoxin. Experimentally, the trap-inducing capacities of *Cooperia*

curticei, *C. oncophora*, *H. contortus* and *O. ostertagi* and of equine cyathostomes are comparable to those of free-living soil nematodes (Nansen *et al.*, 1988). Nematode species with highly motile larvae appear to be the most effective inducers of trap formation but larvae from all species can be rapidly captured in pre-formed traps (Gronvold *et al.*, 1993). A species of oyster mushroom has also been shown to have immobilising effects on several pre-parasitic larvae of *O. ostertagi*, *C. oncophora*, *Oesophagostomum* spp. and equine cyathostomes (Larsen and Nansen, 1991). If fungi are to be used for practical control they must be capable of growing and trapping pre-parasitic stages of nematodes in faeces after they are fed and passed through the gastrointestinal tract of animals. The results of tests to assess survival and viability of various nematode-trapping fungi after passage through animals are controversial. However, *Duddingtonia flagrans* appears to survive well. In practical terms when this fungus, growing on barley grains, was offered to a group of eight grazing calves, the average herbage larval count was reduced by 66% and faecal egg output was reduced by 54% (Gronvold *et al.* 1993). Comparison was made with a control group of eight calves fed fungi-free barley and grazed in a separate pasture. At the end of the experiment the average body weight of the fungi-fed calves was 23 kg higher than the average body weight of the control group.

1.1.6 *Haemonchus contortus*

Description and distribution

H. contortus belongs to the genus *Haemonchus* of the superfamily Trichostrongyloidea of the order Strongylida in the class Nematoda. It occurs in the abomasum of sheep, goats and several other ruminants world-wide but is most important in tropical and sub-tropical areas (Urquhart *et al.*, 1987). The worm is commonly referred to as the large stomach-worm or wire-worm of ruminants and is one of their most pathogenic parasites. The male has an even

reddish colour while in the female, the white ovaries are wound around the red intestine producing the appearance of a 'barber's pole' (Lapage, 1962). The cervical papillae are prominent and spine-like and the buccal cavity contains a dorsal lancet. The male bursa has elongate lateral lobes supported by long, slender rays while the small dorsal lobe is asymmetrically situated against the left lateral lobe and supported by a Y-shaped dorsal ray. The spicules are equal in length and each has a barb at the posterior end. The vulva is covered by an anterior flap in the female which is usually large and fleshy but may be small and knob-like in some specimens (Lapage, 1962).

Life cycle

The life cycle of *H. contortus* is direct. Eggs are passed out in the faeces of infected animals and within 15 to 20 hours, hatch into free-living first stage (L_1) larvae. Females are prolific egg layers; Cushnie and White (1947) estimated the daily output of a single worm to be 9000 eggs. The L_1 larvae feed, develop, grow and moult to the second stage (L_2) larvae. After a further period of activity and growth the third stage (L_3) is attained but the second stage sheath is retained. The whole process can be as short as five days but it may be delayed for weeks or months under cool conditions. The sheathed infective larva is strongly resistant to desiccation and freezing.

Hosts become infected through ingestion. The ingested larvae moult twice in the abomasum as they develop into adults. Just before the final moult they develop the piercing lancet which enables them to obtain blood from the mucosal vessels. The pre-patent period is two to three weeks (Lapage, 1962).

Pathogenesis and clinical signs

In acute haemonchosis anaemia develops rapidly and is progressive (Fourie, 1931), which is seen as a dramatic fall in the packed cell volume (PCV). During the subsequent weeks the PCV usually stabilises at a low level due to a

compensatory expansion of erythropoiesis. However, owing to the continual loss of protein and iron into the gut and increasing inappetence, the bone marrow eventually becomes exhausted and the PCV falls still further before death finally occurs (Dargie and Allonby, 1975). The tolerance PCV level below which the pathogenic effects of haemonchosis begin to appear, is about 28% for Merino sheep (Albers *et al.*, 1990). At necropsy between 2000 and 20,000 worms may be present on the abomasal mucosa which shows numerous small haemorrhagic lesions (Urquhart *et al.*, 1987). However, fewer worms than these can cause death. Charleston (1965) observed that histologically, these petechiae were actually localised areas of glandular congestion. The abomasal contents are dark brown or chocolate coloured owing to abomasal haemorrhage (Fourie, 1931). Charleston (1965) noticed red blood cells (RBC) free in the crypts of the gastric glands on the sixth day after an artificial infection of sheep while Hunter and Mackenzie (1982) noticed streaks of blood in the abomasal contents on the seventh day. Andrews (1942) saw blood in faeces 6-10 days after infection. This is consistent with the expected marked decreases in PCVs which occur at approximately 10 days post-infection (Radhakrishnan *et al.*, 1972). In heavy infections the carcass is pale and oedematous and the red marrow has expanded from the epiphyses into the medullary cavity. In chronic cases caused by small persisting adult burdens of *H. contortus,* anaemia is still the main sign and sub-mandibular oedematous swellings called 'bottle-jaw' are frequently seen. Other swellings may develop along the ventral aspects of the abdomen and the animals become progressively weak and later walk with a swaying gait. Body fat is replaced by a gelatinous tissue and the skin becomes pale and wool falls out in patches. Appetite is variable and although diarrhoea may occur (Lapage, 1962), it is not a common feature of haemonchosis (Charleston, 1965).

In rare cases, very heavy hyperacute infections of up to 30,000 worms occur in which case apparently healthy sheep may die suddenly from severe haemorrhagic gastritis (Urquhart *et al.*, 1987). Allonby and Urquhart (1975)

recorded 35,000 worms in a Merino ewe that had died of this form of haemonchosis in Kenya.

1.1.7 Pathophysiology

In general infection with parasites may cause deaths but more often adversely affects production by impairing growth or causing loss of weight, by altering the host's body composition and by reducing wool and milk production. Parasitism due to helminths is usually also attended by one or a combination of the following: reduced feed intake, anaemia, hypoalbuminaemia and hyperglobulinaemia (Berry and Dargie, 1976; Abbott *et al.*, 1985a).

Reductions in liveweight gain vary with the level of infection, the species of the parasite and the age, nutritional and immunological status of the host (Holmes, 1986). However, there are indications that the most significant economic losses are associated with subclinical infections (Nansen, 1986). Considerable reductions in liveweight gain have been reported in sheep infected with *T. colubriformis*, *O. circumcincta* or *H. contortus* (Allonby, 1975; Sykes and Coop 1976, 1977; Abbott *et al.*, 1986) or with a mixture of several gastrointestinal nematodes (Boag and Thomas, 1973). In addition to body weight changes, economically important alterations in body composition also occur. The most common of these are decreases in the deposition of fat, protein and skeletal calcium and phosphorus together with increased body water as a percentage of body weight (Holmes, 1986). Hence live weight, although easily measured is not always reliable as an index of production. For instance carcass quality in *Fasciola*- infected animals may change independently of weight when there is oedema (Berry and Dargie, 1976).

Gastrointestinal helminths may also adversely affect both the quality and quantity of wool and the inter-relationship between wool production and severity of infection has been demonstrated in sheep with monospecific or concurrent infections of *T. colubriformis* and *O. circumcincta* (Steel *et al.*, 1982).

Although few studies have been conducted in sheep to assess the effect of helminths on milk production, reduced yields have been reported in housed ewes exposed to *H. contortus* (Thomas and Ali, 1983).

Feed intake

For reasons as yet unknown, parasitic infections in general, but more commonly those involving the GIT reduce the voluntary feed intake (reviewed by Symons, 1985). The degree of inappetence varies but is generally related to the level of infection. Most workers quite understandably implicate the lesions produced by the parasites particularly since some of these changes occur in locations housing the receptors concerned with monitoring gut tension, motility and changes in digesta contents (Dargie, 1982a). However, in animals with a given worm load, feed intake often falls progressively with increasing duration of the infection and more markedly in association with poor quality diets especially with regard to protein content (Dargie, 1982a; Abbott *et al.*, 1986). Such findings cannot be explained simply on the basis of variations in the severity of local lesions but could suggest that the clinical condition of the animal as reflected in its haematological and/or plasma protein levels, may also influence the level of feed intake.

Anaemia

Poor production due to parasitism is normally associated with reductions in PCV values with or without decreases in both absolute and relative amounts of plasma proteins. Dargie and Allonby (1975) described three stages in the development of anaemia in *H. contortus* infected sheep. In the first stage the PCV falls progressively but serum iron concentration is normal. In the second stage PCV stabilises, abomasal haemorrhage continues and erythropoiesis is marked. In the third stage there are dramatic PCV and serum iron reductions as erythropoiesis is severely compromised.

Gastrointestinal motility, digestion and absorption

Infected animals frequently show reduced utilisation of digested nutrients relative to parasite-free animals on the same feed intake (Dargie, 1982a; Holmes, 1986). From the limited studies conducted on gastrointestinal motility in parasitised ruminants it can be concluded that infections of the abomasum and small intestines can seriously disturb the normal pattern of gut motility and digesta flow even in the absence of diarrhoea. The general view is that the rate of flow through the gut is reduced partly due to decreased feed intake and partly due to the effects of the parasites themselves.

The impact of alterations in some gastrointestinal secretions on digestion is uncertain. In ovine ostertagiasis and haemonchosis abomasal pH and blood pepsinogen concentrations are often elevated. Increases in pH may be associated with marked changes in the abomasal fluid contents with increased sodium levels and numbers of viable bacteria and decreased potassium and chlorine levels as observed in calves with ostertagiasis (Dargie, 1982a). Gastrin levels in the blood are also greatly elevated in sheep with ostertagiasis (Anderson *et al.*, 1981) even when adult worms are transplanted into the abomasa of uninfected animals (McKellar *et al.*, 1986). This implies that parasites or their secretions have a direct effect on gastrin secretion or activity (Holmes, 1986). In addition, Jones (1983) reported that parasitic infections of the small intestine are associated with decreased activity of a variety of brush border enzymes probably as a result of mucosal damage.

In view of the structural damage and alterations in gut motility and secretions in parasitised animals, there have been numerous attempts to determine whether impaired digestion and absorption are major causes of poor feed utilisation. The results of such studies are difficult to evaluate but in general they indicate that impaired digestion and absorption are not important causes of poor food utilisation and that it is the increased metabolic demand placed on the host by the

parasites' activities that is crucial (Holmes, 1986). Dargie (1982a) concluded that parasites exert their main effect by lowering the efficiencies with which apparently digested and metabolisable energies were used for fat and protein deposition since these were reduced by 30 to 45 % in many pair-fed control studies.

Protein metabolism

A distinctive feature of gastrointestinal parasitism is the loss of proteins into the gut. The proteins are derived from plasma, red cells, exfoliated epithelial cells and mucus (Dargie, 1982a; Holmes, 1986). There is also reduced nitrogen retention which is associated with depressed growth rates and other production effects. The decreased nitrogen retention is in most cases linked with increased urinary nitrogen loss (Parkins *et al.*, 1973; Sykes and Coop, 1976) and is a reflection of the low efficiency with which parasitised animals utilise digested nitrogen (Dargie, 1982a). Conventional nitrogen balance studies do not, however, permit direct assessment of the levels of endogeneous protein loss or the fate of the proteins within the gut (Holmes, 1986).

Mineral metabolism

A number of studies have demonstrated that skeletal growth and mineralisation are impaired in sheep infected with *T. colubriformis* (Reveron *et al.*, 1974; Sykes and Coop, 1976; Coop *et al.*, 1976), *T. vitrinus* (Sykes *et al.*, 1979) or *O. circumcincta* (Sykes and Coop, 1977) often with no overt clinical symptoms of parasitism. There are several causes of impaired skeletal growth in parasitised sheep, for instance poor utilisation of phosphorus (Reveron *et al.*, 1974), and these appear to be related to the level and site of infection.

Water and electrolyte balance

Diarrhoea is a common feature of parasitised ruminants, especially those at pasture. In experimental infections increases in the water content of faeces leading to frank diarrhoea have been reported in moderate to heavy infections in all the important gut nematodes of cattle and sheep except *Haemonchus* spp. In *Ostertagia* infections, the onset of diarrhoea coincides with the maturation of larvae to adults. This is also the time pathophysiological changes such as inappetence, plasma protein losses and negative nitrogen balance are particularly pronounced (Holmes and Maclean, 1971; Parkins *et al.*, 1973) and water turnover is markedly altered (Holmes and Bremner, 1971). Diarrhoea may not necessarily be accompanied by a net increase in water loss since losses in faeces may be compensated by diminished losses in urine.

Host factors modulating pathophysiological responses

The host factors that can influence the pathophysiology of parasitic infections include the genetic background of the host, plane of nutrition and immunological status. There are several reviews on genetic resistance to parastic infections (e.g. Urquhart, 1980; Dargie, 1982b; Kassai, and Sreter, 1992).

Early studies by Whitlock (1949) generally indicated that animals on poor nutrition were less resistant to parasitism than those that were well fed, although the precise reason for this was unknown. Many of the recent studies designed to elucidate the reason(s) have been unsatisfactory either because of inadequate controls or poorly formulated diets (Holmes, 1986). Studies by Abbott *et al.*, (1985a, b, 1986) tried to overcome these shortcomings by using pair-fed controls and a compounded diet that differed only in protein content. The results showed that the protein content of the diet *per se* did not influence the establishment of single primary infections of *H. contortus*, contrary to the suggestion by Preston and Allonby (1978). However, clinical signs of haemonchosis were more pronounced in lambs given the low protein diet despite similar levels of blood

loss. These effects can be influenced by genetic factors since breeds that are particularly susceptible to haemonchosis are more severely affected than relatively less susceptible breeds on low levels of nutrition (Abbott *et al.*, 1985a, b). Such studies illustrate the importance of sub-optimal nutrition in field situations where the problem may be exacerbated by the additional metabolic demands of extensively searching for food (Holmes, 1986).

The age and immunological status of the host may also influence the pathogenesis of nematode infections. More severe effects are more often observed in young ruminants presumably because of immunological immaturity to nematode infections. However, older animals, even though immune to clinical disease may suffer production losses when subjected to a heavy larval challenge (Anderson, 1973; Yakoob *et al.*, 1983).

1.2 IMMUNITY TO GASTROINTESTINAL PARASITES

1.2.1 Immunity, the immune system and its components

The skin and the linings of the respiratory, gastrointestinal and urogenital tracts present formidable physical and chemical barriers to infective organisms and represent a first line of defence against infection. This innate resistance (Staines *et al.*, 1985; Steward, 1986) also has a cellular component involving the polymorphnuclear (PMN) leucocytes, which circulate in the blood and migrate into tissues very quickly in the event of an invasion, blood monocytes and tissue macrophages. The latter cells form the macrophage-monocyte system previously known as the reticuloendothelial system. Unlike the PMN leucocytes which suffer rapid exhaustion, macrophages are capable of sustained effort (Tizard, 1987). The liver, spleen, lungs, kidneys and lymph nodes have large numbers of macrophages (Staines *et al.*, 1985). Natural killer cells and the lysozyme-secreting lachrymal cells are also important in innate immunity. These non-specific host defences are mediated by the complement system for cell lysis and virus neutralisation and interferon which interferes with viral replication (Harlow

and Lane, 1988; Templeton *et al.*, 1988).

Infectious agents that succeed in penetrating these barriers usually elicit the mechanisms of the next line of defence which involves specific, acquired or adaptive immunity. Apart from infectious agents there is an extremely wide range of materials including synthetic substances that can stimulate the specific immune system. Any molecule that can bind to an antibody is an antigen while any molecule that can induce an adaptive response is an immunogen (Harlow and Lane, 1988). These specific host defence mechanisms are provided by a dual system made up of humoral and cellular components.

The humoral response is located in the serum and mucosae and protects primarily against extracellular phases of bacterial, viral and parasitic infections. The response is characterised by the production of antibodies, a group of highly complex structurally related, biologically polyfunctional protein molecules. Antibodies are secreted by B-lymphocytes and can bind specifically to antigens and thus trigger a number of effector mechanisms which culminate in the eventual elimination of the antigens. On the other hand the cellular response is particularly effective against intracellular organisms, which cannot be reached by antibodies, and against cancer cells. Cellular responses are performed by T lymphocytes which, through T-cell receptors, recognise antigen only when it is expressed on the surface of an infected/altered cell in association with a surface marker. These surface markers belong to an important group of molecules known as the major histocompatibility complex (MHC) which were first identified through their ability to evoke rejection of transplanted tissues from donors of the same species (Roitt, 1991). Intracellular organisms can survive inside the phagocytic macrophages by subverting the innate killing mechanisms of these cells. However, the macrophages are still able to process small antigenic fragments from the parasites and place them on the host cell surface. A sub-population of T-lymphocytes called the helper (T_h) lymphocytes, if primed to that antigen, will recognise and bind to the combination of antigen and Class II

MHC molecules on the macrophage surface and produce soluble factors called lymphokines or cytokines. These factors include interferon which activate natural killer cells of the innate immune system and other macrophage-activating factors. Thus the previously subverted microbiocidal activities of the macrophages are activated and the cells are now able to kill the intracellular organisms (Steward, 1986; Roitt, 1991). In general the lymphokines regulate both immune and inflammatory responses (Miller, 1990). Another T-lymphocyte phenotype called the cytotoxic (T_C) lymphocyte can kill infected cells, especially virus-infected cells. Like the T_h lymphocytes these cells recognise antigen only in association with a cell marker, in this case Class I MHC molecules. Through this recognition of surface antigen the cytotoxic cells come into contact with the target cell and kill it. Lymphocytes are organised in lymphoid tissues such as the thymus and the lymph nodes. Unlike the innate immune system, the specific immune system has a 'memory' since the system can be boosted with repeated exposure to foreign molecules.

The ultimate fate of all foreign materials that come into contact with the body's immune system is removal by the phagocytic cells.

1.2.2 Vaccination against haemonchosis

There have been various attempts at immunisation against *H. contortus* with variable results.

Immunisation with normal larvae

In general, exposure to *H. contortus* infection conveys a substantial degree of immunity to re-infection in adult sheep but response to challenge infection is varible in lambs under seven months of age. For example, infection with normal *H. contortus* larvae conferred a considerable degree of immunity in 10 to 12 month old Down lambs, each given a total of 9000 L3 larvae either in two equal doses at a month's interval or in 30 doses of 300 larvae at two-day intervals

(Manton *et al.*, 1962). Concurrently, two to four month old lambs were not protected when similarly exposed to a total of 3000 larvae. It has also been shown that triple oral inoculations with 3000 *H. contortus* L3 three weeks apart conferred substantial protection against challenge infection in four and a half to five month old but not in two and a half month old lambs (Wilson and Samson, 1974). However, conflicting results have been obtained. For instance, Christie and Brambell (1966) induced strong resistance to *H. contortus* challenge infection in two to three month old lambs by first exposing them to several doses of infective larvae followed by anthelmintic treatment before the larvae reached maturity. The level of protection was higher than that obtained in a similar experiment with eight month old lambs (Christie *et al.*, 1964). This was attributed to the more uniform susceptibility of the control group of the young lambs which may, in turn, have been due to the inability of most young lambs to develop an effective immune response after a primary infection. Acquired immunity to *H. contortus* larvae has been shown to wane between six and 12 weeks after challenge and it may be abolished by corticosteriod treatment (Jackson *et al.*, 1988). It has been suggested that animals subjected to prolonged, continuous antigenic stimulation with *H. contortus* may enter a state of immunological exhaustion (Dineen and Wagland, 1966). For example, in one study in three to four month old Merino-Border Leicester cross-bred lambs in which a challenge was superimposed on several sensitising infections of *H. contortus*, the lambs were susceptible. In contrast, where the sensitising infections were terminated by anthelmintic treatment prior to challenge, the lambs were resistant (Wagland and Dineen, 1967); the best response developed in lambs challenged four and eight weeks after termination of the sensitising dose. Conflicting results were obtained by Barger (1988) who reported that untreated Merino lambs naturally infected with *H. contortus* acquired substantial immunity to the parasite at four months of age compared to treated lambs. In another study, infection with 40,000 *H. contortus* L3 combined with the

parenteral administration of larval and adult worm homogenate resulted in increased susceptibility while reducing the infection dose to 20,000 larvae resulted in a measure of protection (Adams *et al.*, 1982). These latter findings suggested that the amount of antigen presented via the abomasal route could have an influence on immunological unresponsiveness.

Differences in the immune responsiveness to *H. contortus* may also be related to breed differences since it has been shown that two to six month old St. Croix lambs can develop high levels of acquired resistance to haemonchosis (Courtney *et al.*, 1985a; Gamble and Zajac, 1992). However, it is likely that in lambs there is slow maturation of immune competence at the mucosal surface and cellular changes have been found to be delayed following challenge in lambs vaccinated with irradiated larvae compared with those seen in similarly vaccinated ewes (Salman and Duncan, 1985).

Injection of fresh or formalin-treated *H. contortus* L_4 and L_5 obtained from the abomasa of infected sheep has also been reported to induce a 70% protection to challenge infection (Silverman and Patterson, 1960). In contrast, exsheathed L_3 induced only a slight immune response when administered subcutaneously while intraperitoneal and intravenous administration elicited no responses (Wilson and Samson, 1974).

Immunisation with irradiated larvae

There have been a number of immunisation trials in sheep with irradiated L_3 of *H. contortus*. Immunisation has mainly involved giving sheep 10,000 irradiated larvae twice orally at an interval of one month (Jarrett *et al.*, 1961; Urquhart *et al.*,1966a, b; Bitakaramire, 1966; Smith and Christie, 1979; Sivanathan *et al.*, 1984), followed by a challenge infection with normal larvae about four weeks after the second immunising dose. In almost all of these studies with Scottish Blackface lambs there was a solid immunity in animals at least seven months old. For example, a 100% reduction in worm burdens was obtained in

vaccinated 10 to 14 month old sheep autopsied 16 days after challenge with 50,000 larvae (Bitakaramire, 1966). In another study, similarly vaccinated two month old Suffolk-Grey face cross lambs were unprotected even when the larval vaccine was combined with parenteral antigen administration but adult sheep were quite well protected by the larval vaccine alone (Smith and Angus, 1980).

In contrast, a much lower level of protection (40%) was recorded in Scottish Blackface lambs first vaccinated at five months of age, but the number of irradiated larvae present in the two doses was not indicated (Ross *et al.*, 1978). Also, Lopez and Urquhart (1967) failed to induce any immunity in seven month old Merino sheep in Kenya with the above regime of vaccination. These sheep were in two groups; those that had prior natural exposure from birth and those that were reared parasite-free. In a subsequent experiment a high degree of protection, with a worm reduction of 98%, was obtained in Merinos maintained worm-free from birth and vaccinated at 24 months of age. Accordingly, it was concluded that East African Merino sheep were immunologically unresponsive up to a point in age between seven and 24 months. From these results it was suggested that immunity was either temporary and largely independent of the existing adult infection or alternatively failed to develop under field conditions because of exposure to infection during the long period of unresponsiveness. In later studies, Benitez-Usher *et al.* (1977) found that a similar immunisation schedule conferred a high degree of immunity in nine or 10 month old Scottish Blackface lambs, which was not altered significantly if the lambs received six spaced doses of normal larvae from 10 weeks of age until the point of vaccination. However, worm-free lambs vaccinated at nine or 10 months of age failed to develop immunity to subsequent challenge if treated with thiabendazole three weeks after each of the two immunising doses. This was in complete contrast to the results of Smith and Christie (1979) who removed vaccine worms with levamisole and achieved strong immunities against challenge infections. Smith and Christie (1979) suggested that this difference was probably related to

the time the vaccine worms are allowed to stay in an animal before removal; the longer the stay the better for the development of immunity.

Vaccination with irradiated larvae has failed to protect lambs less than seven months of age (Urquhart *et al.*, 1966a; Ross *et al.*, 1978; Benitez-Usher *et al.*, 1977). It is this failure that led Urquhart *et al.* (1966b) to investigate the approximate age at which successful immunisation may be achieved. Three groups of lambs were vaccinated twice with 10,000 irradiated larvae and challenged at 13, 21, and 29 weeks of age. Only the oldest group had any evidence of acquired immunity after challenge. In concurrent investigations on the cause of vaccination failure neither the use of Freund's Complete Adjuvant (FCA) nor of *Fasciola hepatica*/FCA (an antigen that has been shown to stimulate production of gamma-globulins) made vaccination effective (Urquhart *et al.*, 1966b). Reducing the vaccine dose to 1,000 irradiated larvae just in case the heavier dose was overloading the antibody producing mechanisms, as shown with *T. colubriformis* infections (Gibson, 1952), made no difference. Any beneficial effect of FCA is doubtful because in other studies in which FCA was administered alone or in combination with *H. contortus* antigen, the results were variable. Thus sonicated adult worms given in FCA subcutaneously imparted some protection while intraperitoneal injection of whole L3 sonicates in FCA increased susceptibility (Adams 1989). Also, single intraperitoneal injections of FCA alone in lambs before, during or after infection reduced worm burdens by 30-55% compared to untreated controls (Bautista- Garfias *et al.*, 1991).

Two other vaccination and challenge regimens introduced by Urquhart *et al.* (1966b) produced no meaningful results. The first consisted of the intra-peritoneal inoculation with 10,000 normal L3, followed 30 days later by oral dosing with 10,000 irradiated larvae then challenge. In the second method lambs were challenged with 20 daily doses of 500 normal larvae after vaccination with two doses of 10,000 irradiated L3.

Immunisation with parasite antigens

Efforts to vaccinate animals with somatic extracts of nematodes have been largely unsuccessful. It has been suggested that the antigens which induce protection are produced in only small quantities by larval but not adult stages (Silverman, 1970). Immunisation of sheep with combined somatic, exsheathing fluid and metabolic antigens of *H. contortus* L3 and L4 have been shown to reduce challenge worm burdens by 50% , while somatic and metabolic antigens of L4 reduced the burdens by 64% (Silverman, 1965). Neilson (1975) attempted to identify functional antigens in the metabolic products released by L3 larvae of *H. contortus* following exsheathment and ecdysis to L4 in *in vitro* cultures. This identification was considered necessary because it was thought that whole worm homogenates either contained insufficient functional antigens, or that such antigens were destroyed during extraction or were masked by the presence of large amounts of non-functional antigens (Silverman, 1965). In later studies, Smith (1977a) found that soluble and insoluble L3 and L4 *H. contortus* antigens in adjuvant did not induce any protection when administered to six month old Scottish Blackface lambs. However, marked serum and mucus IgG antibody responses were stimulated in vaccinated lambs but no mucus IgA antibodies were produced. Earlier, Dineen and Wagland (1966) had suggested two categories of parasite antigens, that is those which provoke the immune responses which affect the survival or fitness of the parasites and those that stimulate immune responses which do not affect parasite survival. The findings of Smith (1977a) probably support this view.

Neilson (1975) fractionated metabolic products by gel filtration and attempted to demonstrate their functional activity by polyacrylamide gel electrophoresis with antisera raised in sheep and rabbits. Three month old lambs were repeatedly injected with up to 0.5 mg of the fractionated antigens with and without adjuvant but failed to resist a challenge infection of 3000 *H. contortus* L3 given a week after the last inoculation. In a similar experiment somatic and excretory

components of *H. contortus* obtained during the development of L3 to L4 *in vitro* were used to immunise lambs less than six months old (Neilson and van de Walle, 1987). On this occasion animals inoculated with a high molecular weight fraction of these extracts developed some degree of resistance (59%) which was similar to that induced (61%) in another group of lambs given a primary infection of 5000 L3 prior to challenge with 10,000 L3. Unfractionated extracts and low molecular weight fractions were completely ineffective as immunogens. Earlier, Ozerol and Silverman (1970) had recovered, fractionated and serologically tested the metabolites derived from *H. contortus* L3 and L4 cultured *in vitro* and found that some of these were capable of stimulating resistance to challenge in lambs. The lambs were immunised with two active components and then challenged orally with 50,000 infective larvae. When compared with untreated controls, the vaccinates excreted 72 and 82% fewer eggs. These results were seen as a positive step towards the development of an effective vaccine against haemonchosis but subsequently Boisvenue *et al.* (1987) failed to find any immunisation potential of a purified, concentrated, serologically active metabolite of exsheathed L3. In three to four month old lambs, three serial inoculations of the metabolite in adjuvant only managed to reduce the fecundity of adult female worms following a challenge infection, the mean total worm counts being similar to those of the controls. Adams *et al.* (1982) found that combining parenteral vaccination of sheep with unfractionated *H. contortus* adult and larval antigens with an anthelmintic-abbreviated infection increased their susceptibility to challenge infection compared with challenge controls. Reducing the sensitising dose from 40,000 to 20,000 larvae yielded some protection.

More recent studies have indicated that 'contortin', a polymeric helical structure which is present in the intestinal cells of *H. contortus* from the early L4 stage is antigenic. Preliminary experiments showed that, depending on the amount administered, contortin afforded a degree of protection to lambs aged

about six months (Munn *et al.*, 1987). Later work showed that contortin obtained from adult *H. contortus* stimulated the development of specific circulating antibodies in younger lambs aged 48 to 150 days. These lambs were also significantly less susceptible to haemonchosis than unvaccinated lambs when challenged a month later with single doses of 20,000 to 25,000 L3. Eighteen of the 19 lambs injected with contortin produced precipitating antibodies and they all survived the challenge infection. In contrast, nine of the 13 challenge control lambs and the immunised lamb which failed to develop antibodies, died of haemonchosis. This protein was deemed useful for vaccination because, being in contact with host blood, it stimulated circulating antibodies in the sheep which bound the protein resulting in the lysis of the adjacent cell plasma membranes of the parasite (Munn *et al.*, 1987). It is of particular interest that this protein appears to protect lambs less than three months old when it has generally been found that lambs less than seven months old are immunologically unresponsive to helminth parasites. Much more recently vaccination of sheep with an integral membrane protein designated H11 reduced egg counts by over 90% compared to unvaccinated challenge controls (Munn *et al.*, 1993; Smith *et al.*, 1993). Cuticular antigens have not proved useful in protection studies (Ihiga, 1991; Boisvenue *et al.*, 1991).

Passive immunisation with transfer factor

It has been shown that transfer factor (TF), a lysate prepared from leucocytes of adult donor sheep, can transfer resistance to infection with *T. axei* and *O. circumcincta* (Ross and Halliday, 1978). The use of TF in *H. contortus*-infected four and seven month old sheep produced 34% and 45% reduction respectively in the number of worms recovered at necropsy following challenge with 10,000 L3 (Ross *et al.*, 1978). In a concurrent experiment another group of four month old lambs were not protected following vaccination with irradiated larvae. This led to the conclusion that TF activity was independent of immune competence.

Mackenzie *et al.* (1982) treated four month old lambs with a combination of transfer factor, adult parasitic antigen and precipitated immunoglobulin from *H. contortus*-infected sheep and achieved a significantly ($p<0.05$) lower worm burden in animals thus treated compared with untreated infection controls.

Passive immunisation with lymphocytes

Lymphocytes leaving the abomasum of sheep immune to *H. contortus* have been shown to transfer immunity between monozygotic twins (Smith *et al.*, 1984). Thus whole lymph or washed lymph cells from three donor sheep infused into their identical co-twin recipients reduced their susceptibility to challenge with 10,000 infective *H. contortus* larvae as assessed by faecal egg counts. Cells from a non-immune donor did not have this effect. Further, washed cells from an immune triplet which was actively responding to repeated challenge infections transferred a secondary local IgA response to a second recipient triplet and resulted in a marked reduction in worm counts compared with a third infectivity control triplet.

Immune exclusion and larval migration inhibition of mucus

It has been observed that exsheathed *H. contortus* L_3 associate with superficial abomasal mucus. In naive sheep 50% of these larvae enter the glandular tissue within four hours of acquisition but in hyperimmune sheep the larvae are excluded from the tissue. This `immune exclusion' is thought to be responsible for the rapid loss of larvae that occurs in immune sheep (Miller *et al.*, 1983). Since IgA can prevent macromolecular uptake by both gastrointestinal and respiratory tract epithelia (Bienenstock *et al.*, 1981) 'immune exclusion' is probably IgA-dependent. If the mechanism also involves hypersensitivity reactions, as has been suggested by Newlands *et al.* (1990), further studies are required to establish the position because Bienenstock *et al.* (1981) contend that IgA acts as a blocking agent in hypersensitivity reactions. Dexamethasone

treatment markedly reduced immune exclusion in treated immune, compared with untreated immune sheep and it is perhaps by this means that the steroid abolishes host immunity to *H. contortus* (Jackson *et al.*, 1988). There are also differences in the composition of mucin between naive and immune sheep after challenge, with substantial depletion of both neutral and acid mucin occurring in naive sheep while immune sheep have reduced superficial neutral mucin and increased acid mucin deeper in the mucosa (Newlands *et al.*, 1990). These mucosal alterations appear to be essential for the development of an effective protective response and it seems unlikely that parenteral immunisation with soluble antigen will promote such alterations. Perhaps it is for this reason that the most effective vaccine schedules against gastrointestinal nematodes to date involve the use of irradiated larvae (Miller *et al.*, 1983). Administration of antigens parenterally are ideal for stimulating IgG responses but do not result in strong secretory IgA or cellular responses which may be required to protect against helminth infections (Cobon and Willets, 1984).

The larval migration inhibition activity of mucus has been demonstrated using an agar-gel based bioassay (Douch *et al.*, 1983). Mucus from the abomasum and small intestines of *T. colubriformis* resistant sheep inhibited the migration of larvae from the gel by up to 93%. The migration of larvae of other trichostrongylid species was also inhibited. Mucus from susceptible and helminth-free sheep did not significantly inhibit migration. Inhibitory activity was associated with components having some properties of slow reacting substances of anaphylaxis (SRS-A), the major constituents of which are leukotrienes (Lewis and Austen, 1981). Faecal samples have been shown to possess larval migration inhibition activity, samples from resistant sheep having a significantly higher activity than those from susceptible or helminth free sheep. It was suggested that the level of the activity in the faeces of sheep undergoing a challenge infection may be a useful indicator of the sheep's resistance status.

1.2.3 Prospects for molecular vaccines

An example of a recombinant vaccine currently in the market is that for human hepatitis B infection (Murray, 1987), which is based on the recombinant expression of hepatitis B virus surface antigen (McAleer *et al.*, 1984). Unlike the situation with this vaccine, where the engineered protective protein antigen had previously been purified from donor blood, the protective immunogen(s) in parasites cannot easily be obtained by purification since parasites cannot be grown *in vitro* in adequate numbers. Hence the immunogen must not only be generated for the first time by recombinant DNA techniques but must also be selected for by screening a range of recombinant products. In addition, parasites of veterinary importance are complex eucaryotic organisms often dwelling on mucosal surfaces where the induction of anamnestic protective responses are problematic, where the immunogenicity of parasite components is poorly understood and where effector mechanisms of immunity are unresolved (Murray, 1987).

Apart from obtaining a parasite gene product by recombinant DNA technology, additional steps would be necessary including the application of new developments in immunology. With peptide vaccines, these steps are likely to include the identification and synthesis of specific parasite peptides that can stimulate T-cells and the incorporation of such epitopes, together with B cell epitopes, in vaccine antigens. They may also include the construction of recombinant virus vectors which can be used to express recombinant peptides. The use of live recombinant vectors could be a useful way of enhancing resistance against parasites (Smith *et al.*, 1986) and this approach has been tried with schistosomes (Simpson and Cioli, 1987). However, it must not be assumed that recombinant viruses will invariably induce protective immunity.

Studies of the MHC in domestic animals and the further development of typing reagents, continue to be important in the development of molecular vaccines against parasites. Recently, cytotoxic T-cell responses to *Theileria parva* in

cattle have been shown to be restricted to infected target cells which share the same Class 1 MHC determinants (Morrison *et al.*, 1987).

The development of molecular vaccines against parasites appears to be possible but substantial long term research over a range of basic disciplines will be required (Murray, 1987).

1.2.4 Target of the immune response in *Haemonchus* infections

Silverman and Patterson (1960) reported that the most susceptible targets for the immune response against *Haemonchus* were L4 and L5 stages. They observed that serum from infected sheep reacted little with exsheathed L3 while it inhibited the motility of exsheathed L4 and L5 within 10 to 12 hours *in vitro*. Altaif and Dargie (1976) also thought that the immune response was directed against the latter two stages. In contrast, when Bitakaramire (1966) vaccinated 10 to 14 month old Scottish Blackface sheep with two doses of 10,000 irradiated *H. contortus* L3 followed by challenge with 50,000 normal larvae and necropsy on different days post-challenge, he found that the immune response was directed against the L3 and L4 stages. These larval stages were stunted and were fewer in number than those recovered from a challenge control group. Smith and Christie (1979) also observed stunting of larval stages when they necropsied *H. contortus* immunised sheep six days post-challenge. Unlike Bitakaramire (1966), however, they did not find a reduction in the numbers of larvae recovered in comparison with recoveries from challenge controls.

1.2.5 Antibodies and cells involved in the immune responses of hosts against nematode infections

In order to survive for a significant period in the abomasum, adult *Haemonchus* either avoid provoking a protective immune response in the host or in some way evade the effects of any response mounted against them (Smith *et al.*, 1982). This survival is nevertheless accompanied by cellular and antibody responses.

Antibodies

Elevation of certain immunoglobulins occurs in serum and/or gastrointestinal mucus when animals are exposed to normal or irradiated larvae of gastrointestinal parasites or their extracts. Exposure to normal larvae of *H. contortus* elicits serum IgG (Smith 1977b; Gill, 1991; Gill *et al.*, 1993) and/or abomasal IgG and IgA responses (Smith, 1977b; Charley-Poulain *et al.*, 1984; Zajac *et al.*, 1990). Similarly, irradiated larvae cause increased levels of serum IgG (Duncan *et al.*, 1978; Smith and Christie, 1978; Smith and Angus, 1980) and abomasal mucus IgG and IgA (Duncan *et al.*, 1978; Smith and Christie, 1978, 1979; Smith and Angus, 1980). Worm extracts or metabolites also cause elevated serum IgG (Smith, 1977a; Munn *et al.*, 1987; Neilson and van de Walle, 1987; Ihiga, 1991) and mucosal IgG levels (Smith, 1977a) although much of the mucosal IgG is probably derived from circulating IgG (Smith, 1977b). Antibody levels increase only slightly after a single infection and IgM responses also occur in serum and/or mucus (Charley Poulain *et al.*, 1984; Zajac *et al.*, 1990; Gill *et al.*, 1993) and it has been shown that worm egg antigens are recognised following infection (Charley- Poulain *et al.*, 1984). Various antibodies have been detected in faeces using an ELISA and in a *H. contortus*- resistant Merino line, both serum and faecal IgA and IgG_1 were positively correlated with resistance after a single infection with 20,000 L_3 (Gill *et al.*, 1993). These antibodies may be involved in immunity to helminth infections (Windon and Dineen, 1981; Gill, 1991; Gill *et al.*, 1993), but their specific roles are poorly understood and in many cases their levels may be elevated without any evidence of protection (Smith, 1977b; Cuquerella *et al.*, 1992).

In general IgA protects mucosal surfaces while IgE is associated not only with parasitic infections but also with allergic reactions (Staines *et al.*, 1985; Roitt, 1991). These two antibody types are likely to play an important role in immunity to helminth infections, but there appear to be no studies on the role of IgE

responses in *H. contortus* infections.

Cells

Various cells have been found to increase in the gastrointestinal mucosa following exposure of sheep to nematode larvae or extracts. Working with *T. colubriformis*, Dineen *et al.* (1978) noted that globule leucocytes in duodenal tissues of infected sheep were negatively correlated with worm counts and therefore positively correlated with resistance. However, this correlation was not strong in a group of sheep that showed marked resistance (responders) to challenge infection after vaccination with two doses of 20,000 irradiated *T. colubriformis* L_3 given two weeks apart. This was taken to suggest that the cells were by-products of a chain of cellular reactions involved in resistance. Douch *et al.* (1986) found that the globule leucocyte was strongly associated with resistance to *T. colubriformis* infection and with increased larval migration inhibitory activity of mucus. In contrast, Smith and Christie (1979) found inconsistent numbers of globule leucocytes in *H. contortus* resistant (vaccinated) and susceptible (unvaccinated) sheep and concluded that these cells are not essential for the expression of immunity. However, more marked increases in mucosal globule leucocytes were shown to occur in adult ewes than in lambs after two infections with *H. contortus* (Salman and Duncan, 1984). Similarly, significantly higher numbers of globule leucocytes were found in immune lambs following two *H. contortus* infections than in challenge or worm-free controls (Zajac *et al.*, 1990).

Eosinophils are also generally associated with parasitic infections. These cells possess anthistaminic properties and nematodes are capable of stimulating histamine release with consequent eosinophil attraction and activity. Increases in eosinophils derived from the circulation were evident as early as four days after *H. contortus* challenge; they then increased over the first 12 days before rapidly declining to pre-infection levels by Day 35 (Hunter and Mackenzie, 1982). In

contrast Charleston (1965) recorded a primary eosinophil response up to 10 days which was followed by a secondary increase at around Day 16. These cells also increased in the abomasal mucosa within five days of challenge in ewes repeatedly immunised with radiation- attenuated *H. contortus* L3 but this increase was delayed in similarly treated lambs (Salman and Duncan, 1985). Eosinophilia in the abomasal tissues has been shown to be associated with the development of L3 to L4 and to peak at the time of the L4 and L5 moults (Hunter and Mackenzie, 1982). The main eosinophil response in the early phases of infection with *H. contortus* therefore suggests that eosinophil activity is directed against the developing larval stages and their initial establishment (Hunter and Mackenzie, 1982) rather than in parasitic expulsion as suggested by Charleston (1965). The enzymes and prostagladins released by these cells (Wakelin, 1978) probably alter the intestinal environment, making it inhospitable to parasites. Eosinophils and mast cells have membrane receptors for IgE and IgG which when triggered cause the release of various substances (reviewed by Miller, 1990). In mice infected with *Mesocestoides corti*, eosinophilia has been shown to be T-cell dependent; it was induced in naive recipients undergoing a primary infection by transferring *in vitro* maintained T-cells following several re-stimulations with soluble crude parasite antigen (Lammas *et al.*, 1987). This transfer was associated with a 40-50% reduction in worm burdens in the recipients undergoing a primary infection. A number of studies indicate that eosinophils can also damage or kill helminth parasites *in vitro* (Butterworth *et al.*, 1975; Mackenzie *et al.*, 1981) by releasing various biochemical substances including enzymes such as perodixases (Tizard, 1987). For example, eosinophils from onchocercosis patients adhered to and immobilised the microfilariae and L3 of *Onchocerca volvulus in vitro* (Brattig *et al.*, 1991). This effect was more pronounced with the L3 than with the microfilariae. The adherence was shown to be antibody and complement dependent but inactivation of complement resulted in some eosinophil adherence to the L3, indicating that there were also

complement-independent mechanisms involved. Adherence of eosinophils to infective larvae of various other helminths, especially in laboratory animal-parasite models, has also been demonstrated (Mackenzie *et al.*, 1977, 1978; Badley *et al.*, 1987). In sheep, peripheral eosinophilia has been positively correlated with resistance to *T. colubriformis* (Dawkins *et al.*, 1989; Buddle *et al.*, 1992) but not to *H. contortus* infections (Gill, 1991).

With regard to lymphocytes, an increase in IgA plasma cells was found in the mucosa of ewes infected and reinfected with *H. contortus* (Salman and Duncan, 1984) or challenged with normal larvae after immunisation with irradiated larvae (Salman and Duncan, 1985). Gill *et al.* (1994) found significantly more IgA containing cells in *H. contortus*-resistant Merino sheep than in random-bred sheep between Days 14 and 35 of infection. There were also significantly more IgG_1 containing cells in resistant than in random-bred lines between Days 14 and 28. It has also been shown that lymphocytes from the abomasum of sheep immune to *H. contortus* can transfer immunity between monozygotic twins (Smith *et al.*, 1984).

It is highly probable that the T_h lymphocytes have a key regulatory role in the generation of non-specific and specific immune responses to parasites (Haig *et al.*, 1989). These workers developed T_h lines from the peripheral blood of sheep undergoing a primary or a secondary infection with *H. contortus* and found that the cells could be stimulated by fractionated larval antigens. The *in vitro* stimulation of lymphocytes in the presence of antigen has been used to assay the response of lambs to *H. contortus* antigens. This test is based on measuring the uptake of ^3H-thymidine by lymphocytes in culture. Other results suggest that primary infection with *H. contortus* provokes a state of selective although not *Haemonchus*-specific, immunological unresponsiveness to nematode antigens (Adams, 1978). Thus while lymphocyte reactivity to extracts of *H. contortus* and *T. colubriformis* was present in lymphocytes obtained from eight to 18 month old worm-free sheep, reactivity was virtually absent in lymphocytes from

infected sheep. This is in contrast to the findings of Haig *et al.* (1989). Lymphocyte reactivity was more marked in lymphocytes from sheep in which infection had subsided naturally or had been removed by anthelmintic treatment, which suggested that this selective immunological unresponsiveness was an adaptation by which *H. contortus* evades host protective immunological responses. Riffkin and Dobson (1979) have also reported that *H. contortus* antigen-induced lymphocyte transformation was absent in neonatal lambs but that lambs gradually developed responsiveness over the first five months of life in the absence of infection. The level of response varied considerably between animals but was heritable and positively correlated with resistance to subsequent primary, secondary and trickle infections. However, in contrast to the findings of Adams (1978) and of Monsell *et al.* (1984) that the lymphocyte response to mitogens, unlike the response to antigen, was unaffected by the presence or absence of infection, Riffkin and Dobson (1979) reported that there were no differences in responsiveness to antigen or mitogen. Lymphocytes derived from worm-free or infected lambs less than nine weeks old were found to be unreactive to *H. contortus* antigen (Monsell *et al.*, 1984; Shubber *et al.*, 1984). Re-infection at 26 weeks of age resulted in only a transient increase in responsiveness in some of the lambs (Monsell *et al.*, 1984). However, this response could be modulated in lambs injected with *H. contortus* antigens early in life or in those born to heavily infected dams (Monsell *et al.*, 1984; Shubber *et al.*, 1984). These *in vitro* lymphocyte transformation studies generally indicate that lymphocytes are reactive to worm antigens but as with humoral responses, the results are very variable.

The importance of other cells associated with *H. contortus* infections such as neutrophils (Fourie, 1931; Salman and Duncan, 1984), mast cells (Salman and Duncan, 1984, 1985; Gorrell *et al.*, 1988; Zajac *et al.*, 1990) and goblet cells is unknown, but mucosal mast cells have been shown to be activated during the spontaneous expulsion of nematodes from the intestine (review by Miller, 1990).

1.2.6 Conclusions

From the available literature it appears that there are many factors involved in immunity to gastrointestinal parasites. These include cells, antibodies, secretions, T-cell products like the lymphokines (Wakelin, 1985; Lammas *et al.*, 1987) and some pharmacologically active substances like prostaglandins (Kelly and Dineen, 1976), histamine (Douch *et al.* 1986; Steel *et al.*, 1990; Zajac *et al.*, 1990) and leukotrienes (Jones *et al.*, 1990). However, the detection of larval migration inhibition activity in gut mucus from resistant sheep which could not be attributed to prostaglandins or to the amines suggests that these substances are indirectly involved in the resistance of sheep to nematodes (Douch *et al.*, 1983). They probably exert their effect by impairing the metabolic activities of the parasites or altering the gastrointestinal function in a way that produces an unfavourable micro-environment (Dineen *et al.*, 1977). The role of each of the aforementioned factors and the ways in which they interact with each other have yet to be fully elucidated.

In vaccination trials, there is little information on the abundance/ immunogenicity of protective antigens, their nature, location, and the developmental stages at which they are expressed most and on any alterations on their biological activities following extraction (Cobon and Willets, 1984). This makes identification of protection-relevant antigens difficult. The mucosal alterations that appear to be necessary for an effective immune response to take place are more likely to be achieved by an immunisation schedule that will introduce immunogens at the site of infestation. The amounts introduced can, however, negatively modulate the responses.

1.3 GENETIC CONTROL OF RESISTANCE

1.3.1 Resistance and resilience

Resistance

Albers and Gray (1986) and Piper (1987) have defined resistance as the ability to suppress establishment and/or subsequent development of a worm infection. Resistance is manifested by a failure of the parasite to become established, the inhibition of larval stages, reduced fecundity of adult females and the elimination of existing infections, that is, 'self-cure' (Riffkin and Dobson, 1979). Resistance may also result in stunting of worms (Kloosterman *et al.*, 1978; Smith and Christie, 1979) and in arrested development of the vulva flap in species which possess this cuticular structure (Waller and Thomas, 1978). These manifestations arise from a combination of innate resistance and immunity which is acquired as a result of increasing age of the host and/or its previous experience of the parasite (Riffkin and Dobson, 1979). Resistance of individual animals should be assessed by means of parasitological parameters such as egg output and worm burden or by immunological responses (Riffkin and Dobson, 1979 ; Albers and Gray, 1986).

Resilience

This is the ability of an animal to maintain a relatively undepressed level of production when infected (Albers and Gray, 1986). It depends on homeostatic mechanisms (Riffkin and Dobson, 1979) and is measured as the relative depression of productivity. This can be assessed by taking the difference in production, for instance live weight gain, over a period of time during which an animal is infected and over a similar period during which the same animal is uninfected. A cross-over design is used; lambs are infected, left for a period and then treated. Production parameters like weight gains and wool fibre diameter, are monitored in the two periods. Concurrently another worm-free group is similarly monitored and it is infected when the first group is treated (Albers *et*

al, 1984, 1987). Standardisation of infection is necessary to evaluate resilience of individual animals (Albers and Gray, 1986). The genetic correlation between resilience and resistance to *H. contortus* was estimated at 0.56 indicating that resistance and resilience may share some common genetic base (Albers *et al.*, 1984). Selection for resistance therefore would only be expected to be partially effective in reducing production losses due to infection if that was the aim.

1.3.2 Evidence for genetic control of disease expressions

Genetic control has been implicated in the expression of most immunological functions. Evidence is principally obtained from two broad approaches: one, the demonstration that immune response (Ir) genes determine the level of reactivity to a variety of synthetic and natural antigens and two, assortive matings in which laboratory animals are selected for and against specific immunological responses (Windon, 1985).

Immune response genes

A number of autosomal dominant genes have been identified which control specific immune responses to distinct thymus dependent antigens (McDevitt and Benacerraf, 1969). Animals possessing an Ir gene are capable of vigorous cellular and humoral responses to the corresponding antigen whereas individuals which are devoid of the gene do not display cellular immunity and their antibody responses are totally or partially deficient (Benacerraf and McDevitt, 1972). Ir genes can exert specific control over the interaction of cells and in addition they appear to modulate specific responses via factors produced by helper or suppressor T cells (Benacerraf and Germain, 1978). Studies in laboratory animals, particularly the mouse, indicate that most Ir genes are closely associated with the MHC. However, not all responses are MHC-linked (Dorf *et al.*, 1974).

Immunological responses

Immunological responses involve antibody production and cellular and phagocytic activities. The most intensively studied immunological function is that of antibody production.

According to a brief review by Windon (1985), work with Biozzi mice in which the animals were selected for their response to various antigens showed that in the low responders, the catabolism of antigens by macrophages was accelerated. This resulted in less time being available for the cellular interactions involved in antibody synthesis. In contrast, slow antigen destruction in the high responders led to the persistence of antigen and hence to elevated antibody levels. In addition the quantity of antigen required for inducing detectable antibody responses was greatly reduced in the high compared with the low responders. The response to selection was generally unspecific and appeared to be associated with macrophage activity while specific cellular activity was unaffected; control was polygenic and realised heritability was in the order of 20%.

Evidence that antibody responses in domestic animals are genetically regulated is derived from limited breeding experiments and surveys of responsiveness within animal populations. For example, Siegel and Gross (1980) bred chickens for increased and decreased antibody production and antibody persistence to heterologous erythrocytes. High antibody titres were associated with greater resistance to most although not all infectious agents. Lie (1977) found the antibody response of young bulls to human albumin to be genetically determined with a heritability of 18%.

Interestingly, genetic regulation can also influence the qualitative antibody response. For instance, Katz and Steward (1975) and Steward *et al.* (1979) bred two lines of mice on the basis of the relative affinity of antibody produced to protein antigens. They showed that antibody affinity is a genetically controlled parameter of the immune response and that this control was independent of that controlling antibody levels. More recently two inbred strains of guinea pigs were

found to differ both quantitatively and qualitatively in their antibody responses following immunisation with excretory/secretory products of *D. viviparus* (McKeand *et al.*, 1994). Similarly, while mice expressing a particular MHC haplotype were more resistant at low doses of *Trichinella spiralis* infection than other haplotypes, these resistant strains were preferentially suppressed at higher doses (Wassom *et al.*, 1984).

1.3.3 Mechanisms of resistance

The mechanisms of resistance to helminth parasites although poorly understood, appear to involve specific immunity and non-specific hypersensitivity reactions (Dineen *et al.*, 1977), and, as already mentioned, it is highly likely that the helper T lymphocytes have a key regulatory role in these responses (Haig *et al.*, 1989).

Considerable attention has been directed at the involvement of genes linked to the MHC in controlling responses to infection. This follows the well established role of MHC gene products in the regulation of T lymphocyte function and the predominantly T-cell dependent nature of anti-helminth immunity (Wakelin, 1985). However, in experimental mouse infections MHC (H-2) involvement has been definitely established or definitely excluded in comparatively few cases. H-2 linked genes exert a clear- cut influence in the response of mice to *T. spiralis*. Their effects can be measured *in vitro* by the level of proliferative responses in lymphocyte cultures or *in vivo* by the variations in the duration of primary and secondary infections and in worm fecundity (Wassom *et al.*, 1983). Inbred mice which differ at the H-2 locus but have the same genetic background or vice versa (congenic mice) were monitored for their response to *Ascaris* excretory/secretory antigens (Kennedy, 1989). It was found that mice with the same MHC but different backgrounds showed identical recognition of antigens while those with different MHC and the same background responded differently. However, the relationship between immune specificity and effector mechanisms is unlikely to

be simple because the strains of mice which react identically to *Ascaris* infection on the basis of antigen recognition may differ in other immunological responses. Thus strains with the same H-2 differed markedly in their IgE and eosinophil responses (Vadas, 1982).

Helminth parasites are complex multi-cellular organisms and they elicit complex immune reactions in their hosts. It could be predicted therefore that resistance to infection would have several components, that genetic control would be polygenic and that inheritance of variations would follow rather complex patterns (Wakelin, 1985). Although inheritance may be complex as in resistance to infection with *Schistosoma mansoni* where many genes are involved (Jones and Kusel, 1985) this is not necessarily the case. Using inbred strains of mice many workers have shown that differences in resistance to infection can be inherited in a simple fashion, the pattern of inheritance indicating that relatively few genes are involved. For instance the inheritance of resistance as a dominant character in F^1 progeny produced by crossing resistant and susceptible parents has been recorded with several parasites among them *Nematospiroides dubius*, *T. spiralis* and *Taenia taeniaeformis* (Wakelin, 1985).

Wakelin (1985) described immune responses to helminth infections and their genetic control and highlighted the following:-

a) Both B- and T- lymphocytes and other accessory cells like antigen presenting macrophages are the cellular components which initiate the immune response to worm antigens.

b) The interaction of these cells is poorly understood but for cell-mediated immunity, the T_h lymphocytes and hence the Class II cell surface antigens are involved. T-cell antigen receptors bind to the worm and MHC antigens expressed on the surface of the presenting cell as one composite structure otherwise the cells cannot recognise the worm antigens as foreign. T_h cells only recognise antigens presented on cells from the same animal or another animal with a shared MHC molecule. This phenomenon is known as MHC restriction

(Anon., 1987). In the humoral response the B-cell's receptor for antigen is a membrane- bound antibody.

c) The above cellular interactions are necessary to provoke the release of a cascade of regulatory lymphokines which regulate the activities of other cells such as macrophages, mast cells and granulocytes.

d) Cellular elements that are influenced directly by genetic factors are T- and B-lymphocytes, the accessory cells and the bone marrow. Elements with products influenced qualitatively and quantitatively by genetic factors are plasma cells, immunogloblins, complement components, macrophages, granulocytes and mast cells.

e) The end products of responses are immune complexes with worm antigen, complement activation, increased production of cellular enzymes and inflammatory mediators, cell proliferation, delayed hypersensitivity and cell-mediated cytotoxicity. These may result in resistance which is achieved either by direct interaction of antibody and complement with the worms, by cell mediated cytotoxicity involving antibody, complement and various cell types and by acute inflammation which affects the worms directly by altering their environment. Not all these responses are beneficial and some may produce undesirable pathology such as granuloma formation.

1.3.4 The major histocompatibility complex (MHC)

Graft rejection is a T-cell dependent immunological response. In the early part of this century a group of genes in mice were found to determine whether or not skin grafts between different individuals were rejected. This group of genes is now known as the major histocompatibility complex and analogous gene groups have been found in all the mammalian species studied including man. Many of the genes which influence immune responses and hence of resistance to disease are located in the MHC. Studies have been carried out mainly with inbred strains of laboratory animals especially mice, and the genes which determine an

animal's ability to respond to various pathogens have been mapped in these animals. These are known as the H-2 genes in mice. Perhaps the most definitive studies to identify and map the H-2 genes were performed by McDevitt and Sela (1965) and Benecerraf and McDevitt (1972). These workers showed that the humoral immune response to synthetic polypeptide antigens was under the control of genes mapped in the H-2 region and that these genes were closely related to the genes controlling lymphocyte activation or the mixed lymphocyte response in mice. These genes were found to map between the K and S regions which was designated the Ir region. All cells of the body in mammals and probably in all other vertebrates have in their membranes a set of proteins which are the products of the genes of the MHC (Staines *et al.*, 1985).

There are many diseases in which one or more of the antigens controlled by the MHC have higher frequencies among the affected individuals than in a control population (Cullen *et al.*, 1984). For instance, Marek's disease in chickens is correlated with the presence of a lymphocyte antigen designated B21 (Briles *et al.*, 1977).

Ruminants have not been closely examined for such associations because a comprehensive range of lymphocyte typing reagents has yet to be developed (Outteridge *et al.*, 1985). In sheep lymphocyte antigens have been described (Ford and Elves, 1974) and the results indicate that up to four loci can be detected two of which are linked and appear to be part of the ovine MHC.

Outteridge *et al.* (1985) also studied the association between two ovine lymphocyte antigens (OLA), designated SY1 and SY2, and responsiveness of sheep to vaccination against *T. colubriformis*. OLA typing antisera were obtained from parous ewes in matings designed to produce high and low responder flocks after vaccination with irradiated larvae. SY1 was found to be present in high frequency (72.2%) on the lymphocytes of high responder rams while in ewes the frequency in high and low responders was 65.7% and 33.5% respectively. A similar association between SY1 antigen and low faecal egg

count was found in random-bred sheep after vaccination with irradiated *T. colubriformis* larvae followed by challenge with normal larvae. It was concluded that SY1 was likely to be part of the ovine MHC which influenced the sheep's immune response to vaccination against this parasite. Subsequently the antigen was subdivided into two and studied as SY1a and SY1b which appeared to be alleles at the same locus (Outteridge *et al.*, 1986). Most sheep with SY1a+1b had low faecal egg counts but a few had very high counts. This suggested that SY1a+1b was associated with the high responder line but not with low egg counts in particular. Although the results were inconclusive the SY1a+1b was present in none of the low responder males. Other workers have not been able to confirm a clear association between OLA type and resistance, for example after selection for resistance to *H. contortus* (Cooper *et al.*, 1989).

The MHC genes comprise the most polymorphic set of genes in the mammalian genome and more than one antigen can be controlled by each H-2 allele in the mouse. Some of these are 'private' antigens coded only by certain alleles while others are 'public' antigens found commonly along several alleles (Wooley and David, 1984)

1.3.5 Genetic variation in resistance to nematodes

Within breed variation

The recognition that there is genetic variation of hosts to helminth diseases goes back to the 1920s. In 1935 Ackert *et al.* reported that some breeds and varieties of chicken were more resistant to *Ascaridia 'lineata'* than others on the basis of worm numbers and size. By selecting the most resistant cockerels and pullets from a flock of chickens a resistant strain of White Leghorns was developed (Ackert, 1937). Warwick *et al.* (1949) selected sheep and goats for resistance against haemonchosis. They found that intense selection on both sides of the pedigree was necessary for meaningful results. This suggested that several genes, each of whose contribution was small, were involved in resistance.

Subsequently, Whitlock (1958) presented data from almost 500 Dorset sheep which confirmed earlier work indicating the existence of an inherited resistance to trichostrongylidosis (Whitlock, 1955a, b). It was found that only 0.02% of progeny from a ram named Violet had PCVs below 20 compared to 32.7% of progeny from other rams and by selection, fairly susceptible and resistant populations were created resulting in an increase of the susceptible population from 32.7% to 61.8%. Whitlock and Madsen (1958) concluded that the 'Violet' factor acted rather like a simple dominant gene and that its expression was modified by various other factors such as the infective dose and possibly nutrition. In later studies Woolaston *et al.* (1990) selected lines of Merino sheep for increased and decreased resistance to *H. contortus* and compared these with an unselected line after about four generations. Following artificial challenge the resistant line had significantly lower *H. contortus* faecal egg counts than the other two lines. Significant differences were also found between all lines in the minimum PCVs following homologous challenge and also following artificial challenge with *T. colubriformis*. This implied that selection for resistance against haemonchosis also resulted in some resistance against *T. colubriformis*. Selection for *Haemonchus* resistance also decreased the periparturient rise in faecal egg counts in ewes (Woolaston, 1992). Cross-protection between nematode species was also obtained by Scrivner (1967) who found that sheep which were resistant to *Ostertagia* spp. by selection were also more resistant to *Nematodirus* spp. and *H. contortus*. Again selection of both sire and dam rather than of sire alone improved progeny performance. However, Windon and Dineen (1984) found that lambs selected for resistance to *T. colubriformis* were not resistant to *H. contortus* whereas they were more resistant to infection with *T. rugatus*, *O. circumcincta*, and *Nematodirus* spp.

These results indicate that it is possible to select for resistance following artificial challenge and substantial progress in selection of Merino sheep for resistance against *H. contortus* (Piper, 1987) and against *T. colubriformis*

(Windon, 1985) has been made within a few generations.

Between breed variation

There are a number of reports on differences between breeds in their abilities to resist gastrointestinal nematodes. For example, Florida Native sheep were found to be more resistant than Suffolk and Rambouillet breeds to both natural and experimental infections with *H. contortus* (Loggins *et al.*, 1965; Jilek and Bradley, 1969; Bradley *et al.*, 1973). Likewise the Scottish Blackface was more resistant to re-infection than the Dorset (Altaif and Dargie, 1978a, b) and the St.Croix than the Florida Native, Barbados Blackbelly and Dorset (Courtney *et al.*, 1985a; Gamble and Zajac, 1992). In Kenya the Red Maasai was also found to be more resistant than the Blackhead Somali, Dorper, Merino, Corriedale and Hampshire Down breeds (Preston and Allonby, 1978, 1979a).

1.3.6 Breeding for resistance

Laboratory animal host-parasite models

For a number of reasons the most important laboratory animal for biomedical research is the mouse. First, mice are cheap to buy and maintain, secondly there are a large number of inbred strains allowing repetition of experiments with proper controls and thirdly, in the case of genetic studies, the short reproductive cycle of the mouse allows rapid investigations (Wooley and David, 1984). Investigation of the role of genetic regulation of parasite burdens in laboratory animals has involved the use of genetically defined inbred strains and the exploitation of variation within outbred populations by selective breeding (Windon, 1985). Several studies, examples of which are briefly described below, have utilised these strains in attempts to define the mechanisms involved in resistance to helminth infections and their ultimate genetic control.

Wakelin (1975a) observed that between animal variation in *Trichuris muris* worm burdens was much greater in an outbred than in an inbred population and

he presented evidence that these differences depended on genetically determined differences in immune expulsion of the parasite. By exploiting this variation, responder and non-responder lines of mice were established from the random-bred population and relevant genetic studies suggested that only a small number of dominant genes were involved in resistance (Wakelin, 1975b). Since the mice from the non-responder line were capable of producing protective antibodies, the genetic defect probably resided in another component of the expulsion mechanism (Wakelin, 1975c).

In another study mice were selected for their 'refractoriness' or 'liability' to primary infection with *N. dubius* over five generations (Brindley and Dobson 1981). The terms 'refractory' and 'liable' described the degree to which individual hosts passively influenced the infectivity of the parasite as distinct from resistance and susceptibility which imply an active response on the part of the host. The liability of parents was positively correlated with that of their progeny indicating that the trait was genetically controlled. The heritability of liability to infection was estimated at about 45% and the high proportion of refractory mice in the second generation demonstrated that the refractory state was dominant to the liability state in this study. In a similar study with the same parasite, lines of mice with high or low faecal egg counts after secondary infection were established (Sitepu and Dobson, 1982). In both studies it was suggested that a small number of genes was involved because of the high heritabilities and rapid interline segregation realised.

Rothwell *et al.* (1978) selected outbred guinea pigs for resistance and susceptibility to single infections with *T. colubriformis*. Substantial interline differences were observed after five generations which were attributed to earlier worm expulsions in the resistant line and this was thought to be genetically determined. Back-cross analysis of two lines of guinea-pigs suggested that genes that map in or near the Ir region of the animal's MHC influence susceptibility to infection by the parasite. However, other genes possibly not linked to the MHC

may also be required for the full expression of susceptibility (Geczy and Rothwell, 1981).

Domestic animals

Feasibility- The demonstration (above) that breeding for resistance is possible in domestic animals does not necessarily mean that such an exercise is attractive for the sheep or cattle industry. Many hurdles will need to be overcome before breeding for worm resistance is shown to be an effective and acceptable method of parasite control (Albers *et al.*, 1984; Albers and Gray, 1986).

Side effects of selection- It is essential to know what other changes in genetic capacity of animals are brought about by the selective breeding for resistance to parasites. For instance, does such selection affect economically important production characters? Only limited information is available on this subject (Albers and Gray, 1986) but Windon and Dineen (1984) found no indication of any adverse effect of selection for responsiveness to *T. colubriformis* on live weight gain or wool growth of sheep. Similarly, Albers *et al.* (1984, 1987) found that there was no correlation between live weight gain of uninfected lambs and their resistance to *H. contortus* challenge. This suggested that breeding for resistance against these parasites would not have unfavourable effects in the production capacity of the sheep although selection for highly productive plant and animal genotypes often results in serious reductions in their resistance to natural parasites (Day, 1974, cited by Riffkin and Dobson, 1979). Also there is evidence that sheep selected for high fleece weight (Howse *et al.* 1992) or for increased litter size, body weight, or fleece weight (McEwan *et al.* 1992) were more susceptible to gastrointestinal parasites than random-bred controls.

If genetic correlations are such that animals shedding fewer eggs gain weight faster, then favourable genetic change in egg output would be indirectly obtained by selection for heavier weight at a given age. Likewise selection for fewer eggs would not adversely affect the animal's weight gain (Leighton *et al.*, 1989).

However, in the study by McEwan *et al.* (1992) a significant positive genetic correlation was found between strongyle egg count and hogget fleece weight which was in contrast to the negative correlations obtained by Albers *et al.* (1987). This latter study also showed that heritability for resilience was too low to allow substantial selection progress by this trait.

In theory, substantial gains in resistance could be obtained by replacing a susceptible with a resistant breed but Gray (1987) argues that in many production systems the immediate improvement in resistance would be associated with highly undesirable changes in meat and wool characteristics.

Evidence currently available indicates that selection for resistance against a single parasite species may also confer a higher degree of resistance to other parasites (Scrivner 1967; Woolaston *et al.*, 1990).

Cost-effectiveness of selection programmes- The cost effectiveness of selection programmes depends upon the economic importance of the disease, genetic variation within the population and identification of genes with major effects. Within the constraints imposed by the agricultural industry, relatively rapid progress is essential for a selection programme to be economically viable (Windon, 1985). Selection could also produce significant genetic change if genetic control is polygenic but moderately heritable (Leighton *et al.*, 1989). Benefits of a selection programme will accrue through increased productivity levels of resistant animals and/or reducing the costs associated with present control methods.

Costs of a selection programme emanate from the identification of genetically superior individuals, that is, assessing the genetic merit or breeding value of candidate breeding animals. In addition, the introduction of an extra selection criterion inevitably reduces selection intensity for existing criteria (Albers and Gray, 1986).

1.3.7 Heritabilities in genetic resistance to worms

The heritability (h^2) of a character is commonly used to quantify its genetic variability. It is expressed as a proportion or a percentage and broadly indicates the proportion of the phenotypic differences between individuals that can be attributed to genetic factors. In its precise sense, however, h^2 is that proportion of the total phenotypic variance accounted for by the additive genetic component (Fincham, 1983; Nicholas, 1993). Phenotypic variance has two main components; variance due to genetic factors and variance due to environmental factors. Within the genetic variance is variance due to additive genetic effects and variance due to dominance. The additive component is due to differences between homozygotes assuming that all these effects do not interact with each other while dominance occurs in heterozygous situations. Heritabilities in populations can be estimated on the basis of correlations between relatives and even without controlled crosses, provided the relationships between individuals are known (Fincham, 1983). Only a few studies have addressed this issue with regard to resistance against worms. Some estimates are presented in Table 1.1.

Table 1.1: Estimates of heritability of host resistance to nematode parasites

Host	Parasite	Heritability (±SE)	Reference*
Mouse	N. dubius	0.48 (0.78)	1**
Mouse	N. dubius	0.49 (-)	2
Sheep	T. colubriformis	0.41 (0.19	3
Sheep	H. contortus	0.29 (0.12)	4
Sheep	H. contortus	0.27 (0.13)	5
Sheep	H. contortus	0.34 (0.10)	6
Sheep	H. contortus	0.26 (0.09)	6
Cattle	mixed infection	0.31 (0.10)	7
Cattle	mixed infection	0.29 (0.18)	8

*1: Brindley and Dobson (1981)

2: Sitepu and Dobson (1982)

3: Windon and Dineen (1984)

4: Albers *et al.* (1984)

5: Piper (1987)

6: Albers *et. al.* (1987)

7: Stear *et al.* (1984)

8: Leighton *et al.* (1989)

** Estimated heritability to infection which defined the host's passive as opposed to active response to infection

1.3.8 Markers of resistance

Markers of resistance would be extremely useful in identifying resistant animals for breeding purposes. However, no useful markers have been developed so far although the following markers have been proposed and/or assessed:

Haemoglobin (Hb) types

Evans *et al.* (1963) associated Hb types with resistance to nematode infection. It was postulated that *H. contortus* uses ingested blood not only as a source of food but also of oxygen. Since Hb types have different oxygen dissociation curves one Hb type would be advantageous to the parasite over another. Some later studies supported the existence of an association between Hb type and resistance to haemonchosis (Jilek and Bradley, 1969; Allonby and Urquhart, 1976; Altaif and Dargie, 1978a, b; Preston and Allonby, 1979b), while other studies found no such association either in mixed infections (Yazwinski *et al.*, 1979) or in *Haemonchus* infections (Courtney *et al.*, 1985a; Kassai *et al.*, 1990). Also, in the study by Altaif and Dargie (1978a) increasing the infective dose of *Haemonchus* abolished this association.

The present consensus is that Hb type is not a reliable marker of resistance.

MHC

There is strong evidence for an association between the MHC gene complex and resistance to nematode parasites and an increasing body of evidence for an association between MHC genes and resistance to a wide variety of diseases justifies further research with respect to parasitic diseases (Albers and Gray, 1986).

DNA polymorphism

The ultimate genetic markers are those which will detect DNA polymorphisms. This requires intensive research into the mechanisms of resistance or resilience

to be able to identify important nucleic acid sequences and construct corresponding DNA probes. Methods by which this may be accomplished have been suggested by Albers and Gray (1986).

Eosinophils

The blood eosinophil response in mammals is T-cell dependent and is often activated during parasitic infections. In some strains of mice the eosinophilia is also controlled by the MHC (Sewell and Vadas, 1983) which could indicate a role for eosinophils as markers of resistance. However, since individual eosinophil counts prior to infection do not provide any clue as to how an animal will behave after infection (Buddle *et al.*, 1992) the usefulness of these cells as markers may be limited.

Periparturient rise (PPR) in faecal egg output

It has been suggested that the presence or absence of a PPR may be a simple, inexpensive marker for resistance in the selection of dams (Courtney *et al.*, 1984). The more expensive method of selection on the basis of response to challenge infections could be reserved for sires, fewer of which are needed. Subsequent results were, however, disappointing since they showed very poor correlations between the PPR of individual ewes and parasite resistance of their progeny (Courtney *et al.*, 1986).

Blastogenic responses of lymphocytes

Riffkin and Dobson (1979) found that pre-infection lymphocyte blastogenic responses to *H. contortus* antigens were negatively correlated with susceptibility to subsequent infection. This observation implied that lymphocyte activation in the presence of antigen could be used as a marker of resistance. Subsequently, responses of peripheral lymphocytes to antigen were determined before vaccination against *T. colubriformis* to examine this possibility (Windon and

Dineen, 1981). However, no significant correlation between stimulation before vaccination and resistance following challenge was observed and the usefulness of lymphocyte stimulation as a marker appears remote at present.

Larval migration inhibition activity in faeces

It has been suggested that larval migration inhibition activity in faeces could be used as an indicator of resistance (Douch *et al.*, 1983, 1986). However, the animals have to be undergoing an active challenge infection for this to be feasible.

Faecal egg counts

Despite the shortcomings of faecal egg counting techniques, faecal egg counts appear to be the most useful markers of resistance to nematodes presently available.

1.3.9 Factors affecting resistance

As already stated the major factors that affect resistance to gastrointestinal parasites include the age of animal with lambs below seven months of age showing increased susceptibility (Manton *et al.*, 1962; Benitez-Usher *et al.*, 1977; Smith and Angus, 1980; Ihiga, 1991), genetic constitution which is related to the ability to actively acquire resistance (Stoll, 1929; Whitlock, 1955b; Gamble and Zajac, 1992) and nutrition (Clunies Ross, 1932; Whitlock, 1949; Preston and Allonby, 1978; Abbott *et al.*, 1985a). Observed genetic differences imply that some breeds are generally more susceptible than others and it has been shown that malnourished animals show increased susceptibility compared to well fed individuals. Immunosuppression may also affect resistance by depressing the immune response. For example, dexamethasone treatment abolished the superior resistance exhibited by the progeny of a resistant sire (Presson *et al.*, 1988) and prevented immune exclusion of *H. contortus* larvae in

the abomasum (Jackson *et al.*, 1988).

1.3.10 Methods of assessing resistance

Studies that demonstrate genetic control of natural disease resistance in domestic animals are highly dependent on the methods used. Important considerations include the nature of exposure to the pathogen, the method used to assess resistance and the type of genetic analysis used.

Exposure to a pathogen may be natural or experimental. In experimental studies it is easy to standardise the exposure and the dose of pathogen used for challenge is chosen to maximise differences between resistant and susceptible animals while approximating a situation of natural exposure (Templeton *et al.*, 1988).

Resistance and susceptibility may be graded according to the severity of disease induced in the animal or according to isolates of the pathogen or both. If isolation is the criterion used, relative differences may be recorded qualitatively (i.e. isolation of pathogen implies susceptibility) or quantitatively (i.e. isolation of a minimal number of organisms indicates resistance). If severity of disease is chosen for assessing relative susceptibility, additional genetic mechanisms are likely to be measured since three classes of animals are involved in such a study. The three groups are infected and diseased, infected and disease-free and uninfected and disease-free. Thus one would be measuring genes that allow or prevent infection (resistance) in addition to those that allow or prevent disease manifestations (resilience) in infected animals (Templeton *et al.*, 1988). This type of assessment is common in helminth diseases.

Different types of studies are also possible. Family studies are designed to specifically monitor the inheritance of a trait through two or more generations and to allow the study of dominance and recessiveness and of the number of genes influencing a specific trait. On the other hand population studies are designed to identify an association between two or more traits and to determine their frequencies in a population. All types of genetic analyses are based on the

assumption that animals can be identified as resistant or susceptible (Templeton *et al.*, 1988).

1.3.11 Influence of resistance on epidemiology

Epidemiology is concerned with the occurrence patterns, by time, place and hosts, of disease in populations and the factors that influence these patterns (Lilienfield and Lilienfield, 1980).

There appear to have been no long term studies on the effect of genetically resistant hosts on the epidemiology of helminth diseases in either cattle or sheep (Barger, 1989). However, Gibson and Everett (1977) compared infective larval populations on a paddock grazed by sheep with acquired resistance to *O. circumcincta* with another grazed by naive sheep of the same age. Peak larval populations were more than three times higher in the latter paddock. Six month old worm-free lambs were subsequently grazed on the two paddocks and those in the second paddock acquired much higher burdens than those in the first.

If resistant sheep carried fewer worms which laid fewer eggs as shown for instance by Tetzlaff and Todd (1973) who reported a 72% reduction in the number of eggs laid by individual *H. contortus* females in premunised compared with challenge control sheep, then animals would be exposed to fewer larvae at pasture. Albers and Gray (1986) made a predictive model of faecal egg output reductions to be expected from resistant sheep as selection progresses, depending on the number of genes controlling the trait and the genotype (whether homozygous or heterozygous resistant) of the sire. When a major gene is available, selection progress is much faster than when resistance is polygenically controlled and hence faecal egg output declines faster in the former case.

1.4 OBJECTIVES OF THIS STUDY

Since haemonchosis is perhaps the most important parasitic disease of sheep and goats in subtropical and tropical regions of the world and there has been a rapid increase in human population in these areas, there is a demand for a matching increase in food production. With the increased population pressure on existing farming areas, agriculture is becoming more intensified with consequent increase in stocking rates. This increase in stocking rates has created new problems and intensified existing problems, including the occurrence of parasitic disease. Although acute haemonchosis can result in death, of more significance, perhaps, is the insidious effect of chronic haemonchosis which reduces weight gain and carcass quality especially when nutrition is also poor.

Current control measures rely heavily on anthelmintics and are likely to continue to do so at least in the near future. However, anthelmintics are expensive in terms of material and labour costs and there is an increasing population of parasites which are resistant to broad spectrum anthelmintics. There are additional concerns on the possible effects of the anthelmintics on the environment. All these factors emphasise the need to develop other control methods which will be alternatives or adjuncts to chemoprophylaxis. One such method is breeding for resistance against parasites since there is ample evidence that genetic variation plays an important role in such resistance. Unfortunately, this method is generally considered when all other methods have failed or are unsatisfactory (Dolan, 1987).

Breed and individual variations in the resistance of sheep to haemonchosis are well recognised (Piper, 1987; Zajac *et al.*, 1990), although little is known about the underlying mechanisms. However, since lines of sheep with increased resistance or susceptibility can be produced by selective breeding (Warwick *et al.*, 1949; Woolaston *et al.*, 1990) the trait is heritable (Whitlock, 1955b). Current evidence has it that the genetic variability amounts to around 30% of the total variation in host resistance to nematode parasites (Albers and Gray, 1986),

a level that should allow effective selection for resistance (Nicholas, 1993).

The present study was initiated to examine in more detail the previously documented resistance to *Haemonchus* in the Red Maasai sheep of Kenya (Allonby, 1975; Preston and Allonby, 1978, 1979a). It was hoped that the results would provide a foundation on which to base future control strategies against haemonchosis. The following were the aims of the study:-

* To confirm the superior resistance of Red Maasai sheep to *H. contortus* infections compared with other breeds using both natural and experimental infections

* To monitor the haematological, biochemical and serological changes with infection and relate them to resistance

* To compare more closely the resistance of the Red Maasai to that of the Dorper which is increasing in popularity in Kenya

* To investigate whether there are age related differences in resistance between the Red Maasai and the Dorper

* To examine any within breed differences in resistance in the Red Maasai and the Dorper

CHAPTER TWO

2. MATERIALS AND METHODS

2.1 PARASITOLOGICAL METHODS

2.1.1 Modified McMaster egg counting technique

A modification of the McMaster egg counting technique devised by Gordon and Whitlock (1939) was used to detect and count nematode eggs in faeces (Bairden, 1980). Three grams of faeces from a sample taken directly from the rectum was weighed and transferred into a plastic pot. The faeces were homogenised with 42ml water and the resulting suspension passed through an ordinary sieve to remove coarse materials. The filtrate was thoroughly mixed and a 15ml aliquot transferred into a flat bottomed tube which was centrifuged at 1000rpm for 5min. The supernatant was discarded and the remaining faecal pellet broken up by rotary agitation (Whirlmixer, Scientific Industries). The tube was re-filled to its former level with saturated sodium chloride solution, covered with the thumb and inverted six times. Thereafter a volume sufficient to fill the two chambers of a McMaster slide (Weber Scientific Instruments, Middlesex, England) was quickly withdrawn from the tube and transferred to the slide. The total number of eggs within both ruled areas of the slide were counted and the results multiplied by 50 to give the number of nematode eggs present per gram of faeces.

2.1.2 Faecal cultures and recovery of infective larvae

Infective *H. contortus* larvae were obtained by culturing faeces from sheep monospecifically infected with a locally isolated strain of the parasite. Isolation was accomplished as follows: adult female worms were obtained from infected, freshly slaughtered sheep at an abattoir 20 km away from the institute. The worms were finely cut with a scalpel blade to release the eggs. The resulting sediment was cultured in helminthologically sterile sheep faeces; for

sterilisation, the faeces were wrapped in aluminium foil and heated to over 80^{0}C on a hot plate. The few larvae thus obtained were administered intra-ruminally to worm-free sheep using a hypodermic needle. After patency, faeces from the sheep were cultured and the animals re-infected.

The faeces were collected overnight using a faecal bag and harness. The faeces were incubated in 250 ml wide-mouthed containers at 26^{0}C for 10 days in a compact incubator (Leec Ltd., Nottingham, England); the lids were not tightened to allow for aeration of the cultures. Larvae were recovered by a Baermann technique as follows: the containers were removed from the incubator. Warm water at about 37^{0}C was added and the containers left to stand for 3 hours after which the contents were then passed through a coarse mesh sieve (250u) or an ordinary sieve to remove the faecal pellets. The filtrate was passed through double milk filters (Maxa Milk Filters, A. McCaskie Ltd., Stirling) held on a Buchner funnel-conical flask assembly which was connected to a small vacuum pump (Stanhope-seta Ltd., Surrey, England). The filters were then removed and placed on the top of a urine jar in contact with warm water contained in the jar; the larvae migrated through the filters and settled at the bottom of the jar. Larvae were pipetted out and stored in water at 4^{0}C in culture flasks (Nunclon, Roskilde, Denmark).

2.1.3 Enumeration of infective larvae for infections and the infection procedure

Larvae for infections were stored for a maximum of two weeks and were checked for activity and counted a day prior to use.

A 50ul aliquot was withdrawn from a larval suspension after gentle but thorough shaking and transferred to a microscope slide in several small drops. If the concentration of larvae in the drops was too high for easy counting, the larval suspension was suitably diluted with water. The number of larvae in six 50ul aliquots were counted under a compound microscope (Biomed Leitz,

Portugal) and the average calculated and multiplied by 20 to get the number per ml of larval suspension. A note was made of the number of any exsheathed larvae present but these were not included in calculating the larval dose since they usually constituted less than 5% of the total larvae present. Each dose was withdrawn with an automatic pipette into a round bottomed centrifuge tube; thorough mixing preceded each withdrawal.

At infection, the contents of one tube were mixed and emptied into the mouth of a well restrained sheep. The tube was rinsed twice with water and the rinsings also given to the sheep.

2.1.4 Post-mortem worm recoveries

Sheep carcasses were opened and the entire gastrointestinal tract removed from the body cavity after putting pairs of ligatures just posterior to the pyloric sphincter and at the small intestine/large intestine junction. A third pair of ligatures was put between the omasum and the abomasum. The abomasum, small intestine and large intestine were then separated by cutting between each pair of ligatures. The abomasum was split longitudinally in a bucket and the mucosa washed with a slow stream of water. The washings were made up to 2L from which a 10% sample (200ml) was taken, transferred into a 250ml wide mouthed jar and formalinised for subsequent estimation of worm counts. The small intestine was similarly washed and a 200ml sample taken for worm counts. The entire abomasal and small intestinal samples were examined for worms using a dissecting microscope.

2.1.5 Mucosal scrapings and digests

A modification of the method described by Herlich (1956) was used to digest abomasal scrapings. The mucosa of the washed abomasum was gently scraped with a knife and the scrapings divided into two. One half was subjected to digestion in 200-250ml pepsin/HCl for six hours at 40-42^{0}C. A litre of the

digestion fluid was made up of 10g pepsin (Sigma Chemicals, Ltd.) and 30ml concentrated HCl (BDH Chemicals) in distilled water (Bitakaramire, 1966). The digests were made up to 2L and a 200ml sample taken and formalinised for examination later.

The other half of the abomasal scrapings was kept frozen for later immunological studies.

2.1.6 Worm length measurements

In the experiments where worm lengths were measured, only unformalinised intact adult worms were used. After taking a 200ml sample for worm counts from the 2L of abomasal washings, the remainder was allowed to sediment. Some of the worms obtained from this sediment were measured and the rest were used for antigen preparation. Since it was not always possible to measure worms on the day of necropsy, the abomasal sample containing the worms was in some cases frozen at -20^{0}C for measurements later. After thawing the abomasal sample at room temperature, worms were separated from the ingesta and placed in PBS. Six to ten intact females and a similar number of males from either fresh or frozen samples from each animal were measured. Each worm was placed on a microscope slide and straightened with a dissecting needle. The length (in mm) was taken with a ruler.

2.1.7 Assessment of the number of eggs per female worm

In the experiment where eggs per female worm were assessed, six intact adult females from the unformalinised abomasal washings from each lamb were placed in a 5ml glass tissue grinder and 2ml of saturated sodium chloride solution added. The worms were ground to release the eggs. The resulting suspension was thoroughly mixed using a pipette then quickly transferred into the two chambers of a McMaster slide and the eggs counted. The number of eggs in the initial 2ml volume and hence in the six females was calculated and from

this, the number of eggs per female was obtained.

2.1.8 Larval identification

Larvae were identified with reference to the key provided by Dunn (1978).

2.2 HAEMATOLOGICAL METHODS

2.2.1 Determination of packed cell volume (PCV)

PCVs were determined by the microhaematocrit method (Hawksley and Sons Ltd., W.Sussex, U.K.). Blood was collected by jugular venepuncture into evacuated tubes containing EDTA (Becton Dickinson, Vacutainer Systems, England). The blood sample was mixed on a roller mixer (Coulter Electronics, U.K.) and a capillary tube filled to between half and two thirds. One end of the tube was then sealed before being spun on a micro-centrifuge for 4-6 min. The PCV (in percentage) was measured directly on a PCV reader.

2.2.2 Eosinophil counts

A sample of EDTA blood was diluted 1:9 with Carpentier's eosinophil counting solution (2ml 2% aqueous Eosin Y, 3ml formaldehyde saturated with calcium carbonate and 95ml distilled water) as outlined by Dawkins *et al.* (1989). The eosinophils were enumerated in a haemocytometer (improved Neubauer, Hawksley, U.K.) and all the cells in the nine squares in each chamber were counted. By taking into account the volume of the chambers and the dilution factor, the number of eosinophils in one ml of blood was calculated.

2.2.3 Haemoglobin typing

Haemoglobin typing was carried out using Paragon haemoglobin electrophoresis kits and instruments (Beckman Instruments, U.S.A.). EDTA blood, either fresh or stored for up to four weeks at 2-8^{0}C was suitable for the test. The blood was centrifuged at 2000 rpm for 15 min to separate cells from plasma. The plasma and buffy coat were removed and the red blood cells (RBCs) washed once by re-

suspending them in 10 volumes of normal saline (0.85% NaCl), centrifuging this suspension and removing the supernatant. With a disposable pipette a drop of the RBC suspension was placed in a centrifuge tube and 15 drops of Paragon haemolysing reagent added; the contents were then thoroughly mixed. A pre-prepared Hb gel was unsealed and placed on a paper towel. The gel was blotted gently with gel blotter and a template with sample applicator slots appropriately aligned on the gel as recommended by the manufacturer. The template was gently rubbed with a finger to ensure a proper seal. A 3-5ul sample of haemolysed RBCs was applied across each template slot. After five minutes following the application of the last sample the template was gently blotted with a new blotter and discarded. The gel was then placed onto a gel bridge assembly, aligning positive and negative sides of the gel with corresponding positions marked on the assembly. The assembly was then placed into a Paragon elecrophoresis cell and cover, the cell inserted into the power supply and the gel electrophoresed at 150v for 25 min. Thereafter the gel was removed from the cell, placed on a gel frame and then placed in a Paragon drier until just dry. The dried gel was then passed sequentially through paragon blue stain (3 min), acetic acid solution I (2 min), acid-alcohol solution (2 min) and acetic acid solution II (2 min). The gel was removed from the last solution and excess solution was wiped off it. Subsequently the gel was placed in the gel drier until completely dry. A sample from a sheep known to be Hb type AB was always included in each gel to serve as a marker for distinguishing between different Hb types.

2.3 BIOCHEMICAL METHODS

2.3.1 Determination of total serum protein and urea

Total protein and urea concentrations in serum were determined using a Vettest 8008 (Vettest S.A., Neuchatel, Switzerland).

2.4 SEROLOGICAL METHODS

2.4.1 Extraction of parasite antigen

H. contortus L_3 were checked under a compound microscope to ensure that the majority were alive. If a large proportion were found to be dead the larvae were passed through a Whatman no.4 filter paper and re-baermannised to remove the dead larvae. At least three million larvae were transferred into a centrifuge tube and 3ml of sodium hypochlorite (BDH Chemicals) with 4% available chlorine added to exsheath the larvae. The tube was left standing for 1min then topped up with sterile PBS and centrifuged at 2000 rpm for 5 min. The supernatant was removed, sterile PBS added and the larvae re-centrifuged. This was repeated two more times to remove all the sodium hypochlorite. After the last wash, as much of the PBS as possible was pipetted out of the tube and the larvae transferred to a 5ml glass tissue grinder. A small amount of 2M Tris-HCl buffer pH 8.3 containing protease inhibitors (Appendix 1) was added. Protease inhibitors are also effective bacteriostats and thus eliminate bacterial contamination during extraction (Neilson and van de Walle, 1987). The larvae were ground over ice and centrifuged in a micro-centrifuge at 13,000 rpm for 20min. The supernatant was carefully removed, avoiding the superficial lipid layer and the sediment then re-centrifuged. The protein content of the soluble antigen in the final supernatant was estimated by a UV. detection method by reading absorbances at 260 and 280 nm (Harlow and Lane, 1988). The antigen extract was stored in eppendorf tubes at -20^{0}C.

Adult *H. contortus* worms were obtained from infected sheep at slaughter, separated from the abomasal contents and transferred into petri dishes containing PBS. After three washes in sterile PBS the worms were homogenised in a similar manner to the larvae except that a 30ml tissue grinder was used first before using the finer 5ml grinder.

2.4.2 Enzyme linked immunosorbent assay (ELISA)

Immulon, flat-bottomed microtitre plates (Dynatech, Alexandria, U.S.A.) were coated with 5ug/ml of crude *H. contortus* adult or L_3 antigen diluted in carbonate- bicarbonate buffer pH 9.6 (Appendix 2). Each well except those in the first column was coated with 100ul of the antigen solution. Coated plates were covered and incubated at 4^0C overnight. Between steps the plates were washed three times with PBS containing 0.05% Tween 20. Four percent skimmed milk in PBS was used for blocking, followed by addition of test serum diluted 1:60 with 2% skimmed milk in PBS. The test serum was added in duplicate while positive and negative control sera were added in quadruplicate. The second antibody diluted to 1:1000 was anti-sheep IgG cojugated to the enzyme horse radish peroxidase and the enzyme's substrate was o-phenylaminediamine (Sigma Chemicals Ltd.) in citrate buffer (Appendix 3). 5ul of 30% hydrogen peroxide was added for every 10ml of the substrate solution just before use. No conjugate was added to the wells in the first column.

Except for the substrate step where incubation was at room temperature for 5-10 min, the plates were incubated for 30 min at 37^0C. The reaction was stopped with 15% sulphuric acid. Each well received 100ul of each reagent. Plates were read at 492nm on a Multiskan Plus Version 2.2 ELISA reader.

2.5 WEIGHING OF SHEEP

Where sheep were weighed a standard sheep weighing crate was used.

2.6 STATISTICAL ANALYSES

Mixed model repeated measures analysis of variance to test the effects of various factors were carried out with the GLM programme on the SAS package (SAS Institute, Cary, North Carolina). Egg counts, total worm counts, eosinophil counts and ELISA O.D. values were logarithm-transformed to stabilise the variance before analysis. The egg count and total worm count data were transformed in the form $\log_{10}$ (EPG or total worm count +10) while eosinophil

counts and O.D. values were transformed to $\log_{10}$ (eosinophil count or O.D.+1).

CHAPTER THREE

ASSESSMENT OF THE RESISTANCE OF FOUR SHEEP BREEDS TO NATURAL AND SUBSEQUENT ARTIFICIAL *HAEMONCHUS CONTORTUS* INFECTION

3.1 INTRODUCTION

Ovine haemonchosis is an endemic helminth disease of considerable economic importance in tropical and sub-tropical regions of the world and in Kenya the disease has been shown to depress weight gains by up to 30% in infected Merino lambs (Allonby, 1975). Generally, control measures for important helminth diseases of ruminants depend on the use of anthelmintics coupled with various rotational grazing schemes. However, in many parts of the world there are practical difficulties with such schemes and this, together with widespread anthelmintic resistance in the case of *Haemonchus*, has directed attention to possible alternative strategies. One such alternative is to develop potential genetic resistance of the host against the parasite.

Work done about 20 years ago in Kenya showed that the Red Maasai breed was more resistant to haemonchosis than several other sheep breeds after experimental (Castellino, 1976; Preston and Allonby, 1978) or natural (Preston and Allonby, 1979a) infections. This work was prompted by the observation that in flocks of Red Maasai sheep kept under traditional management systems faecal strongyle egg counts were generally low, anaemia was uncommon and clinical cases of acute haemonchosis extremely rare (Preston and Allonby, 1979a). Between breed differences in resistance to haemonchosis have been documented on many occasions with other breeds. For example, Florida Native sheep were more resistant than Rambouillets (Radhakrishnan *et al.*, 1972; Bradley *et al.*, 1973), Scottish Blackfaces than Dorsets (Altaif and Dargie, 1978a, b) and St. Croix than cross breeds and Dorsets (Courtney *et al.*, 1985a; Gamble and Zajac, 1992). There are also within breed differences and Whitlock (1955b) found that

certain individuals within a flock of sheep were better able to resist natural *H. contortus* infections than others and could pass on that ability to their progeny. Earlier, Warwick *et al.* (1949) selected individual animals on the basis of their resistance to haemonchosis and compared the survival rate of lambs from selected dams and sires with those from unselected parents over a nine year period. The differences in survival rates between the two groups were highly significant. It was also found that selection on the basis of one resistant parent had little influence on the resistance of the progeny.

In the present study, between breed variation in resistance was assessed in groups of four sheep breeds after both natural and subsequent artificial *H. contortus* infections. The breeds involved were the Romney Marsh, Dorper, Blackhead Persian (or Somali) and the Red Maasai. The aim was to confirm earlier published reports that the Red Maasai was resistant to haemonchosis as a preamble to investigations on the possible mechanisms involved. The results obtained would also help in determining a long-term strategy on the possibilities of exploiting such resistance as a method of controlling the disease.

3.2 MATERIALS AND METHODS

3.2.1 Animals

Castrated male animals which were 10-15 months at the start of the experiment were used. The Romney Marsh was introduced to Kenya for improved wool and meat production. It is a polled, longwooled breed mainly kept in the Molo area of Nakuru district. The animals used in this experiment were bought from the Government-owned Agricultural Development Corporation (ADC) Nyota farm in Molo. The Dorper breed was developed in South Africa from the Dorset and the Blackhead Somali and was introduced in Kenya in the early 1960s mainly for meat. It is a polled and hairy or coarsewooled breed which is becoming popular in many parts of the country. The experimental group was purchased from Ol Pejeta Ranch in Laikipia district. The Blackhead Somali is a fat-rumped hair

sheep thought to have originated in Somalia. The breed now inhabits the largely semi-arid north-eastern region of Kenya and the group used in this experiment came from pastoralists in Garissa district. The Red Maasai is an indigenous breed of Kenya and Tanzania. Although mainly found in the open grasslands within the Rift Valley, sheep with many of the characteristics of the breed are also seen in many other parts of the country. It is characterised by short reddish brown to almost black coarse hair with a light undercover of wool and in general is fat-rumped. Males are horned or polled but females are usually polled. The Red Maasai sheep used in this experiment were bought from ADC Mutara Ranch adjacent to Ol Pejeta Ranch in Laikipia.

3.2.2 Experimental design

Twenty sheep of each breed were brought to the National Veterinary Research Centre (NVRC), Muguga. They were not given any anthelmintic treatment at this time and after a quarantine period of two weeks they were grazed together for a month in order to contaminate four adjacent paddocks with nematode eggs. These paddocks had not been grazed by sheep for over a year. Fifteen of the 20 sheep of each breed were then treated with fenbendazole (Panacur^{R}, Hoechst) at 10mg/kg body weight before each breed was set-stocked on one of the paddocks. For security reasons all the sheep were housed together in concrete floored accommodation at night. The sheep were weighed and faecal, EDTA blood and serum samples obtained weekly for one year from the 15 treated sheep in each group. The untreated sheep were left to continue contaminating the pastures. At the end of the grazing period, surviving sheep in the experimental groups were dosed again with fenbendazole, housed and after two weeks each animal was infected orally with 10,000 *H. contortus* infective larvae. Four worm-free wethers were kept together with the experimental sheep as extraneous infection controls. Faecal samples for egg counts and EDTA blood for PCV determinations were taken twice weekly for eight weeks. Serum was taken

weekly for serological analyses using an ELISA.

Total worm counts were carried out on any sheep that died during the course of the experiment. At the end of the experiment, all the experimental animals were necropsied for worm counts.

3.2.3 Feeding

While indoors, the wethers were fed on hay and supplemented with a commercial concentrate. Water was available *ad libitum.*

3.2.4 Methods

The parasitological, haematological, serological and statistical methods used are described in Chapter 2.

3.3 RESULTS

3.3.1 Natural field challenge

Mortalities

Twelve Romneys, two Dorpers and four Blackhead Somali died during the natural challenge period in the paddocks and a fifth Blackhead Somali disappeared without trace while grazing. Nine of the Romneys, the two Dorpers and two of the four Blackhead Somali died of haemonchosis. The cause of death of the remaining animals was not clearly established.

Total worm counts

The mean total abomasal worm counts in the 13 animals which died of haemonchosis during this part of the study are shown in Fig.3.1 and the individual animal counts, in Appendix 4. Small intestinal worms consisted mainly of *Trichostrongylus* spp. together with a few *Nematodirus* and *Strongyloides* spp.

Faecal egg counts

In the Romney Marsh group, mean faecal egg counts rose gradually, with fluctuations, to reach a peak of 9200 epg after 25 weeks (Fig.3.2a). The egg counts then declined sharply to 2700 epg in one week followed by a further gradual fall to 1100 epg 7 weeks later (Week 33). From Week 33 the egg counts rose again to attain a peak of 14,000 in Week 41 followed by a second sharp fall to 4000 epg in Week 43. A third peak of 11,600 epg was observed in Week 47 again followed by a sharp decline to 1200 epg the following week. At the end of the experiment the mean count was 983 epg but, by that time only three animals in this group were still alive.

In the Red Maasai egg counts fluctuated below 2000 epg (Fig. 3.2b) with peaks of 1230 epg in Week 7 and 1400 epg in Week 12. The highest mean count of 2000 epg was recorded in Week 51.

Counts in the Dorpers rose to a peak of 5200 epg in Week 9 (Fig 3.2b) then fluctuated markedly between 2000 and 5000 epg until Week 25. The egg counts remained below 2000 epg for the following 12 weeks before rising to approximately 5000 epg in Week 41; there were then marked fluctuations until the end of the experiment.

In the Blackhead Somali egg counts rose to a peak of 7200 epg in Week 9 (Fig.3.2b). After an intervening fall to 3200 epg one week later a second peak of 6400 epg occurred in Week 12. This was followed by a sharp fall to 2300 in Week 15. From then until Week 43 the counts fluctuated between 1300 (Week 40) and 3100 (Week 28). After Week 42 egg counts fell from 2000 to the lowest count of 450 epg in Week 45 before rising to 4400 epg in Week 46. In the remaining six weeks the counts fluctuated between 1400 and 3000 epg. The egg counts in the Dorpers and the Blackhead Somali followed a similar pattern.

A comparison between the four breeds in terms of cumulative mean egg counts is shown in Fig. 3.3. The build up of nematode eggs was remarkably similar in the Dorper and Blackhead Somali up to Week 19. Initially the build up was

higher in the Dorper and the Blackhead Somali than in the other two breeds, consistent with the initial rapid rise in egg counts observed in these two breeds. Thereafter the output of the Dorper became higher than that of the Blackhead Somali but the trend remained similar. In the Romneys there was a steady increase and between Week 24 and Week 34 the cumulative counts were almost the same as those of the Dorpers. Thereafter the counts of the Romneys rose rather sharply above those of the Dorper and plateaued at a higher level over the last six weeks. In contrast, cumulative mean counts in the Red Maasai remained much lower than those of the other three breeds and the change from one week to the next was remarkably constant.

Self-cure, as assessed by the criteria of Preston and Allonby (1979a) of an 80% reduction in group mean egg count over a three week period occurred once in the Dorper and twice in Red Maasai (Appendix 5).

The statistical analyses examined the influence of breed, animal within breed, day of sampling and the interaction of breed with time. The results (Table 3.1) showed that there was a highly significant ($p<0.0001$) breed effect. The effect of animal within breed was also highly significant as was the breed by time interaction.

PCVs

The mean PCVs in the Romney Marsh group was 37% in Week 1 but fell with time to 26% in Week 6 (Fig.3.4a). This was followed by a rise to 32% in Week 11 then a gradual fall to 22.5% in Week 24. Subsequently the PCVs rose to 28% but after Week 35 there was another fall to the lowest mean PCV recorded of 20% in Week 45. Thereafter there was an apparent rapid rise to 32% at the end of the experiment.

In the Red Maasai there was an initial fall from 38% in Week 1 to 32% between Weeks 6 and 9 (Fig.3.4a). Thereafter the values fluctuated between 30% and 37%.

The mean PCV in the Dorpers fell from 34% in Week 1 to 28% in Week 8, followed by a steady rise over the subsequent five weeks to attain the original value of 34% in Week 13 (Fig 3.4b). Thereafter the PCVs fluctuated between 27% and 33%.

The mean PCV in the Blackhead Somali fell from 34% in Week 1 to 26% in Week 8 (Fig 3.4b). Subsequently a steady rise to 31% in Week 13 occurred. In the remaining period the values varied between 26% and 33%.

The PCV levels and changes in the Dorper and the Blackhead Somali reflected similar changes in faecal egg output in these two breeds.

The results of statistical analyses (Table 3.1) showed highly significant breed differences. Individual animal variation, variation with time and the breed by time interaction were also highly significant.

Weights and weight gains

The Romney Marsh sheep maintained their mean weight between 30 and 33kg during the first 11 weeks of the experiment (Fig.3.5a). This was followed by a gradual fall to 26kg in Week 22, then a rise to 34kg in Week 34. Thereafter there was a gradual fall to 26.6kg in Week 46. Cumulative mean weight gains (Fig.3.5b) show that this breed suffered a substantial loss of weight. The net loss was 4.9kg (15.6%) from 31.5kg in Week 1 to 26.6 kg in Week 46. However, the least square mean weight loss which takes into account the missing values was 6.7kg. Only nine animals were remaining at the last weighing in Week 46 but by the end of the experiment in Week 52 an additional six animals had died.

The Red Maasai gained 6.9kg (21.3%) from a mean of 32.4kg in Week 1 to 39.3kg in Week 46. The Dorpers gained 8.2kg (32.3%) from 25.4 to 33.6kg while the Blackhead Somali gained 7.5kg (45.2%) from 16.6 to 24.1kg. The corresponding least square means were 6.7, 7.5 and 7.2kg respectively. During the last weighing there were 15, 13 and 10 animals in the Red Maasai, Dorper and Blackhead Somali group respectively.

Serum antibody responses

Although there were no marked changes in ELISA O.D values with time, higher responses were consistently observed in the two indigenous compared with the two exotic breeds (Fig.3.6) and differences due to breed, individual animal, time and breed by time interaction were all highly significant (Table 3.1).

Table 3.1: Results of analysis of variance of faecal egg count, PCV and ELISA O.D. data of Romney Marsh, Red Maasai, Dorper and Blackhead Somali wethers exposed to a natural *H. contortus* infection

Source	Probability		
	EPG	PCV	ELISA O.D.
Breed	0.0001	0.0001	0.0001
Animal(breed)	0.0001	0.0001	0.0001
Time	0.0001	0.0001	0.0015
Breed*time	0.0001	0.0001	0.0006

Model: PCV or $\log_{10}$(EPG+10) or log (O.D. + 1)= breed animal(breed) time breed*time. Parentheses indicate animal nested within breed while * indicates breed by time interaction

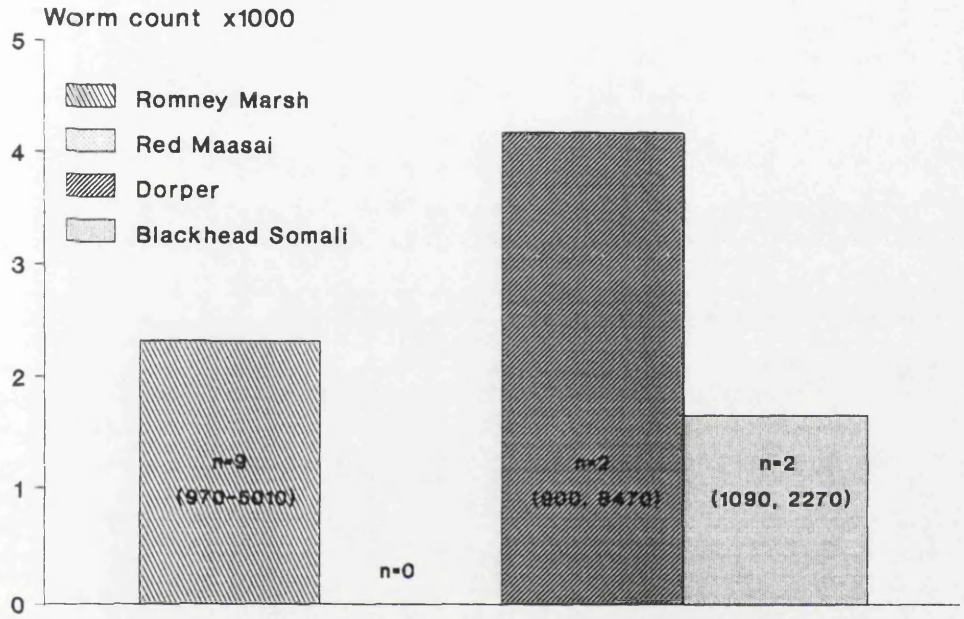

Fig.3.1: Mean adult *H. contortus* worm counts (range) of wethers of four sheep breeds which died of haemonchosis

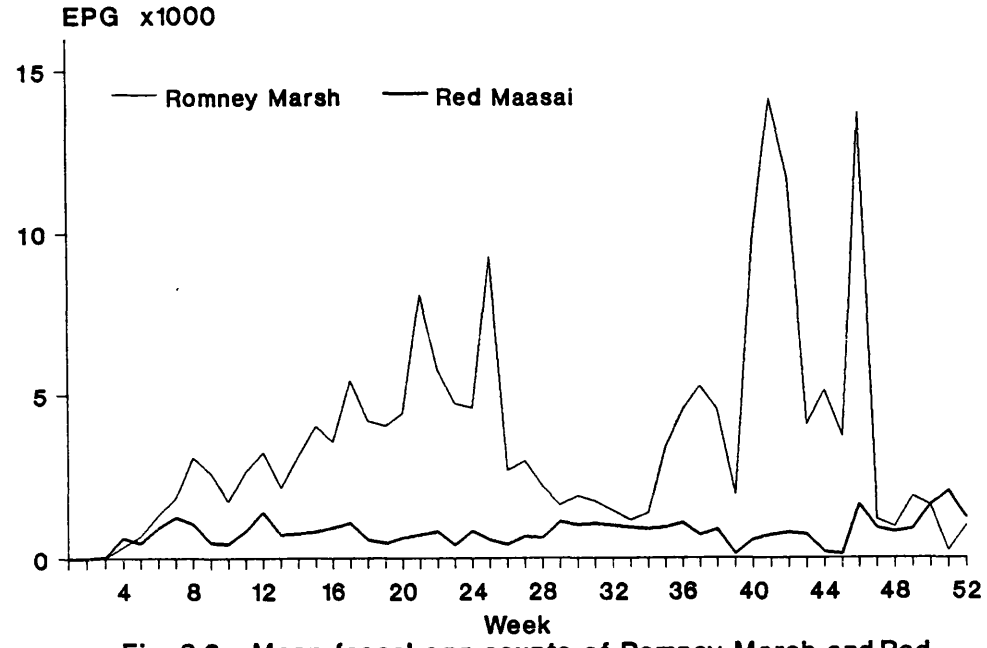

Fig. 3.2a: Mean faecal egg counts of Romney Marsh and Red Maasai wethers exposed to natural *H. contortus* infection

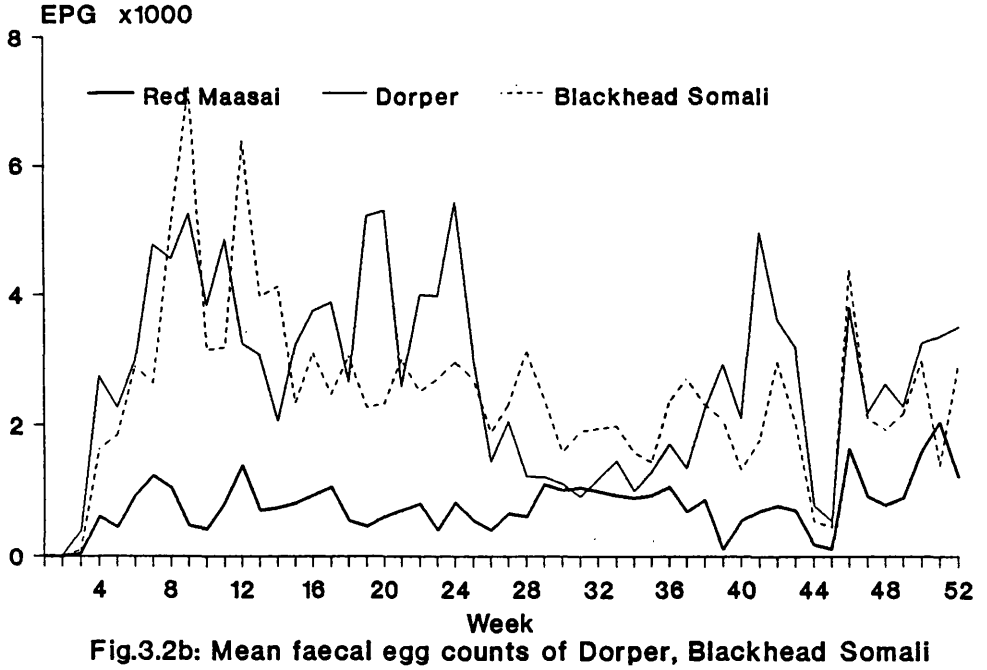

Fig.3.2b: Mean faecal egg counts of Dorper, Blackhead Somali and Red Maasai wethers exposed to natural *H. contortus* infection

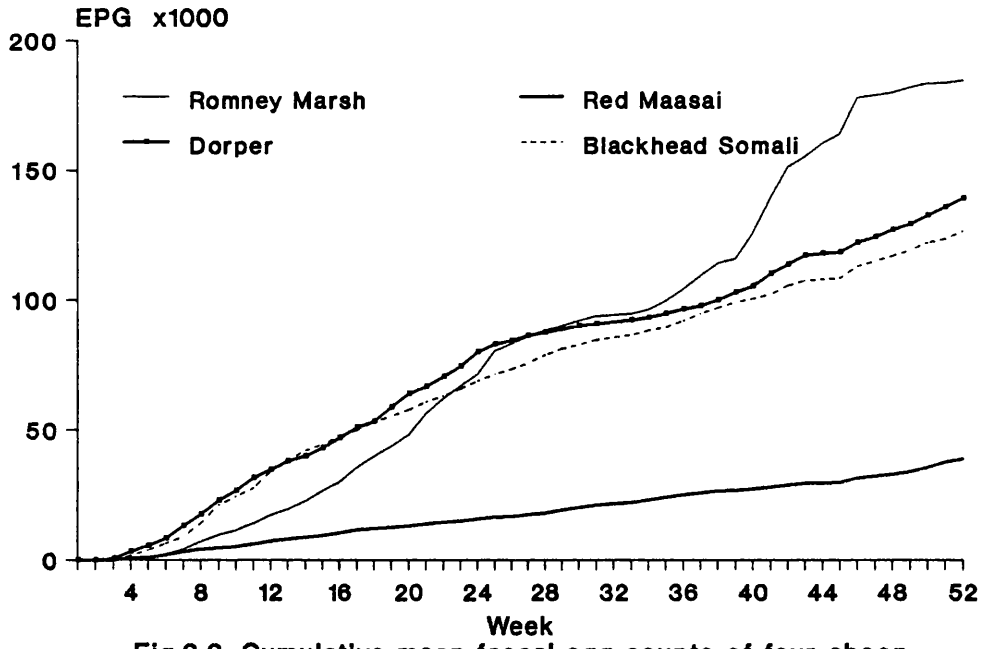

Fig.3.3: Cumulative mean faecal egg counts of four sheep breeds exposed to natural *H. contortus* infection

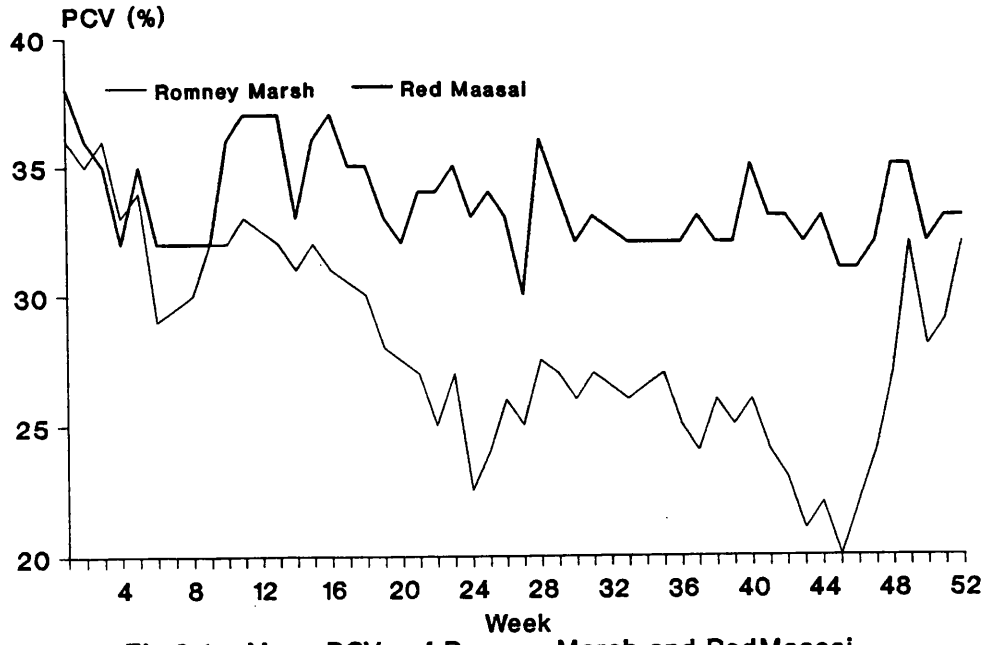

Fig.3.4a: Mean PCVs of Romney Marsh and RedMaasai wethers exposed to natural *H. contortus* infection

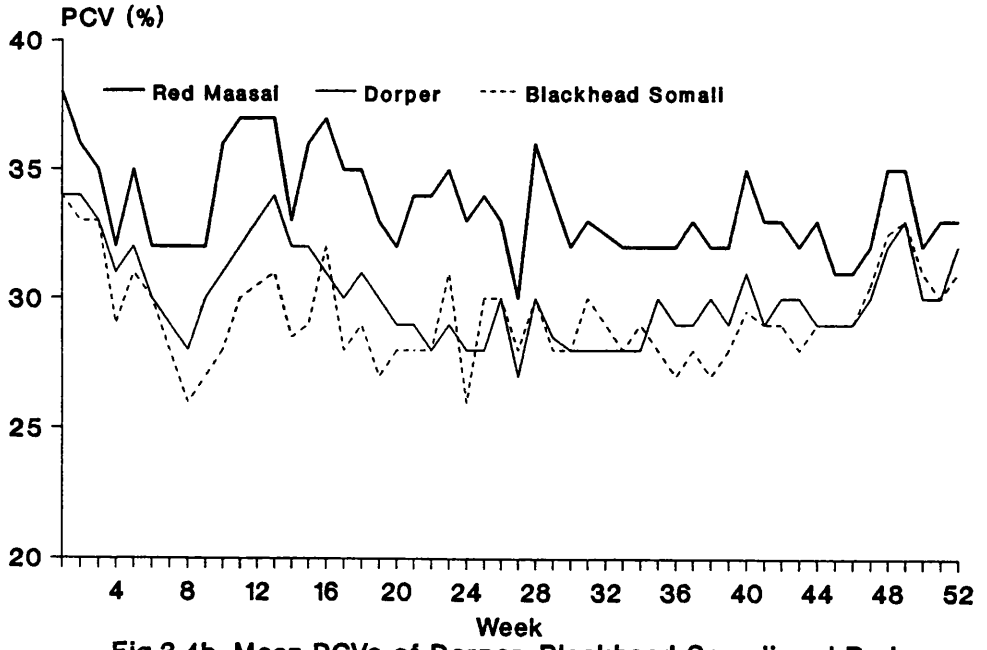

Fig.3.4b: Mean PCVs of Dorper, Blackhead Somali and Red Maasai wethers exposed to natural *H. contortus* infection

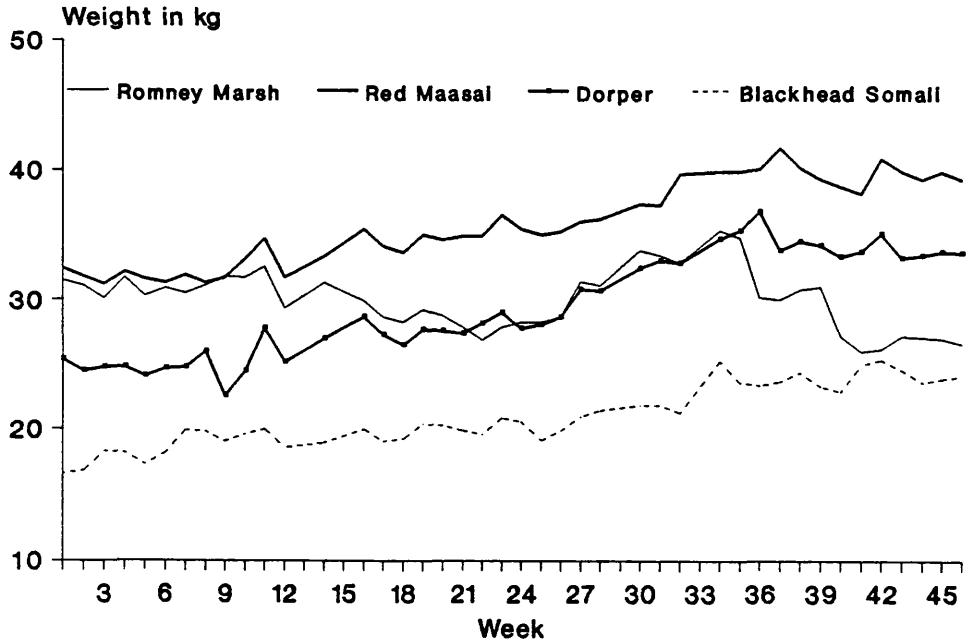

Fig.3.5a: Mean weights of wethers of four sheep breeds exposed to natural *H. contortus* infection

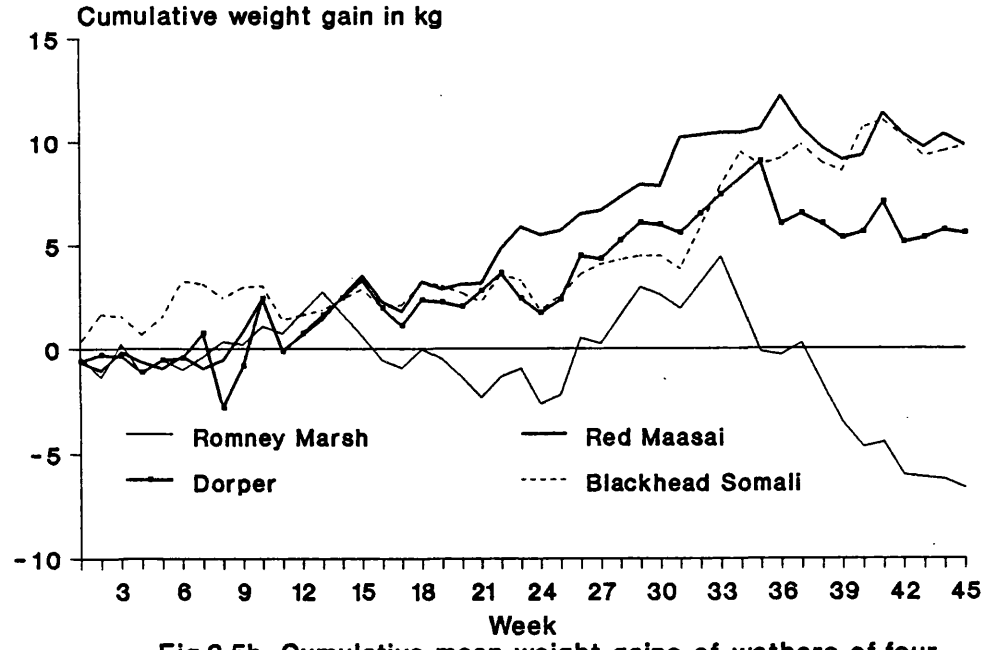

Fig.3.5b: Cumulative mean weight gains of wethers of four sheep breeds exposed to natural *H. contortus* infection

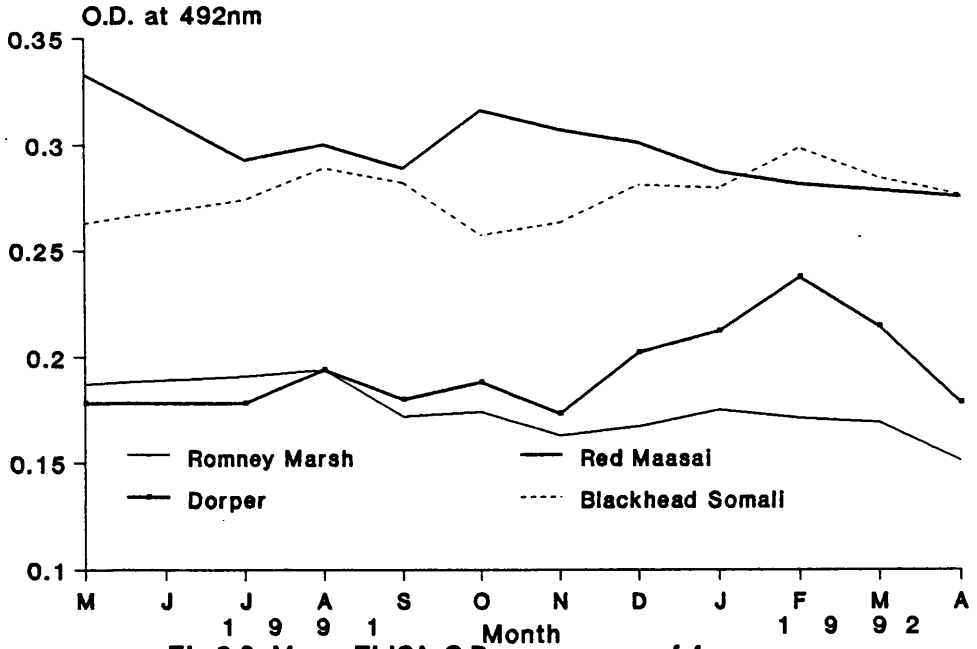

Fig.3.6: Mean ELISA O.D. responses of four sheep breeds to *H. contortus* L3 antigens

3.3.2 Experimental indoor challenge

Mortalities

One Dorper and one Romney Marsh died of pneumonia during the indoor part of the experiment.

Faecal egg counts

The four extraneous control animals remained negative during the experiment.

After infection the surviving Romney Marsh wethers attained a mean peak egg count of 6900 epg on Day 28 (Fig.3.7a). After falling to 3300 epg the following week, the egg counts rose again to 8000 epg on Day 39. At the end of the experiment the average count had risen to 12550 epg. In contrast, the mean egg counts in the Red Maasai remained relatively low, increasing slowly from 300 epg on Day 25 to 1900 epg on Day 53.

The pattern of egg output in the Dorpers and the Blackhead Somali was similar (Fig.3.7a). In the Dorper the mean egg counts rose to a peak of 2150 epg on Day 31, fell to 1300 epg on Day 35 and rose again to a higher peak of 5100 epg on Day 42. The count was 5560 epg at the last sampling. In the Blackhead Somali, the first peak of 2400 epg occurred on Day 28 which was followed by a fall to 940 epg on Day 35 then by a second higher peak of 5100 epg on Day 42. The last count was 4300 epg.

When the results were statistically analysed and the effects of breed, animal within breed, time and breed by time interaction tested, only the breed by time interaction was not significant (Table 3.2).

PCVS

The PCVs fell with time in all four breeds but to varying extents (Fig. 3.7b). In the Romney Marsh the PCVs fell from a mean of 33% (Day 14) to 21.5% (Day 56), a difference of 11.5 while in the Red Maasai the PCV fell by 4 from 35% to 31%. The PCV fell from 31% to 25% in the Dorper and from 32% to 24% in the

Blackhead Somali.

When the effects of breed, animal within breed, time and breed by time interaction were tested they were all highly significant (Table 3.2).

Total abomasal worm counts

The Red Maasai had a lower mean total worm count than the Dorper and the Blackhead Somali groups and the two remaining Romneys (Fig.3.8). The differences in total worm counts were, however, not significant.

Table 3.2: Results of analysis of variance of faecal egg count and PCV data of Romney Marsh, Red Maasai, Dorper and Blackhead Somali wethers infected with a single dose of 10,000 *H. contortus* L_3

Source	Probability	
	EPG	PCV
Breed	0.0008	0.0001
Animal(breed)	0.0001	0.0001
Time	0.0001	0.0001
Breed*time	0.0649	0.0001

Model: PCV or $\log_{10}$(EPG+10)= breed animal(breed) time breed*time

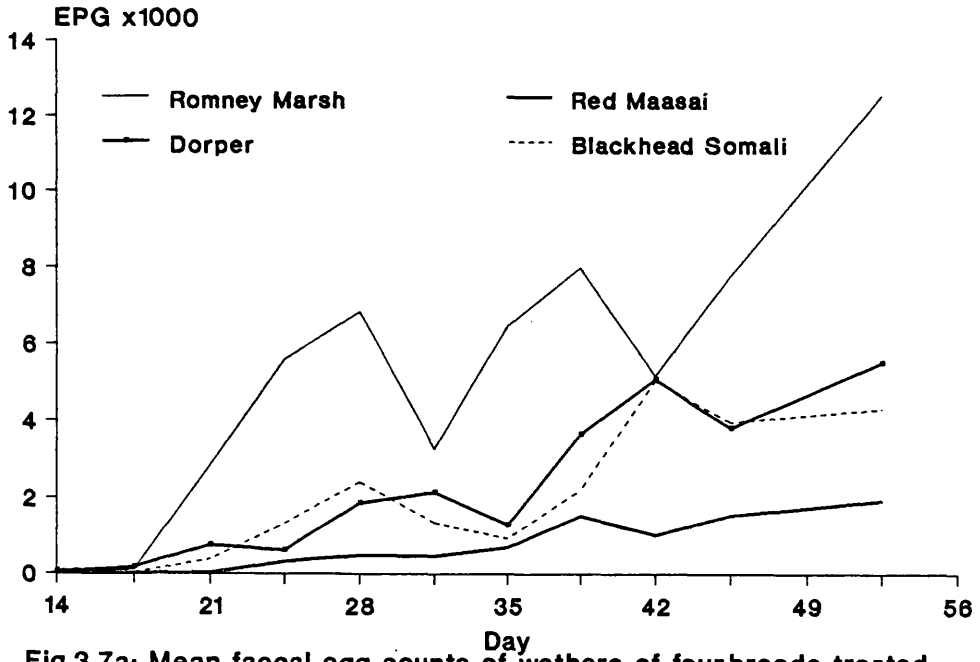

Fig.3.7a: Mean faecal egg counts of wethers of four breeds treated after natural infection and re-infected with 10,000 *H. contortus* L3

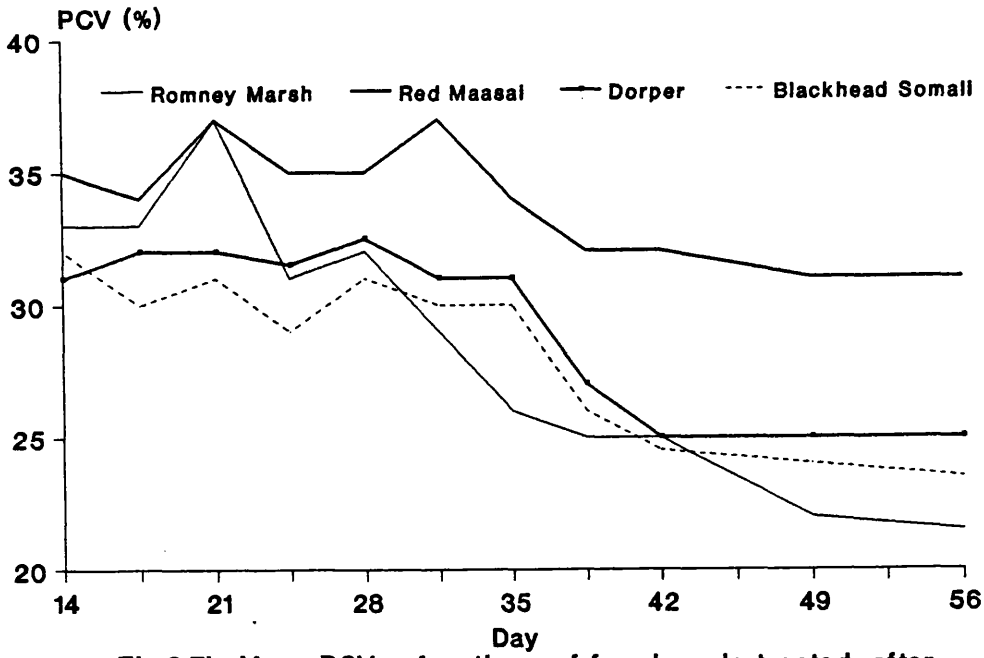

Fig.3.7b: Mean PCVs of wethers of four breeds treated after natural infection and re-infected with 10,000 *H. contortus* L3

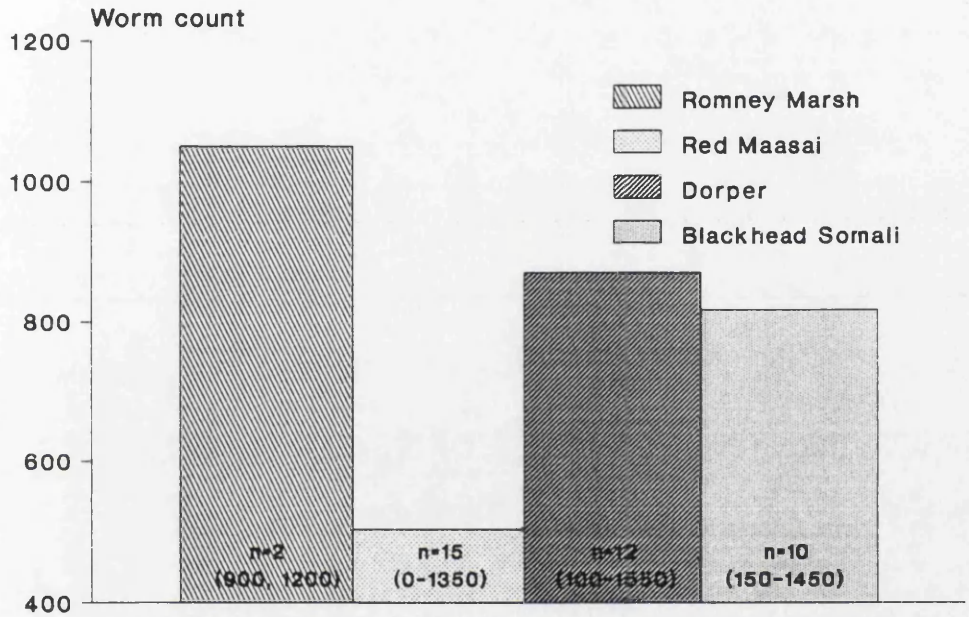

Fig.3.8: Mean worm counts (range) of wethers of four sheep breeds infected with 10,000 *H. contortus* L3

3.4 DISCUSSION

In the field component of this study, egg count results and mortality rates confirmed the earlier findings in a similar field study by Preston and Allonby (1979a) that the Red Maasai is more resistant to haemonchosis than the Dorper and the Blackhead Somali. The results also showed that the Red Maasai was more resistant than the Romney Marsh. While in the Red Maasai the mean weekly egg count did not exceed 2000 epg in 52 weeks, counts went up to 14,000 epg in the Romney Marsh. Over the same period, maximum counts of 5200 and 7200 epg were recorded in the Dorper and the Blackhead Somali respectively. In addition no Red Maasai died while nine Romneys, two Dorpers and two Blackhead Somali died of haemonchosis.

Self-cure, based on the criteria of an 80% reduction in egg count within a three week period (Preston and Allonby, 1979a) occurred twice in the Red Maasai and once in the Dorper. Although there were other occasions when dramatic falls in the egg counts occurred in all the breeds suggesting self-cure, these counts occurred to a lesser degree or over a longer period (Appendix 5). In the study by Preston and Allonby (1979a) spanning a period of two years, faecal egg counts in Red Maasai wethers grazing a contaminated pasture fluctuated between 0 and 550 epg. In contrast, the egg counts in Merino ewes set-stocked with the wethers ranged from 60 to 2000 epg and while self-cure occurred four times in the Red Maasai it occurred only twice in the Merino. Six of the 60 ewes died of haemonchosis while all of the 10 Red Maasai wethers survived. The mean total abomasal worm counts of four of the wethers and four of the ewes necropsied at the end of the experiment were seven and 214 worms respectively. In another experiment described in the same paper 10 Red Maasai wethers were compared with 10 wethers of each of the Blackhead Somali, Merino, Corriedale, Dorper and Hampshire Down breeds. Over a two year period, a mean egg count of over 300 epg occurred on only three occasions in the Red Maasai and all of the 10 Red Maasai wethers survived to the end of the study. In contrast, all the 10

Hampshire wethers died within 26 weeks of exposure and six of the Corriedales, four of the Merinos, three of the Dorpers and three of the Blackhead Somali also died of haemonchosis during the study.

Of the four breeds in the present study the Romneys were the most susceptible to *Haemonchus*. However, in America, this breed has been reported to be more resistant to *O. circumcincta* infection than the Rambouillet, Hampshire, Shropshire and South Down on the basis of faecal egg counts (Stewart *et al.*, 1937). There are also *T. colubriformis*- resistant lines of Romneys established by selection (Buddle *et al.*, 1992). The American investigators assumed that the higher degree of resistance in Romneys was due to natural selection and selective breeding in Kent, their original *Ostertagia*-endemic habitat. While breed comparisons in terms of resistance are usually relative since there are no set critical egg counts or worm burdens by which resistance is judged, epidemiological considerations may be relevant here. A study carried out by Dinnik and Dinnik (1958) on the bionomics and survival of *H. contortus* L_3 in pasture in Kenya showed that certain highland areas of the country were likely to be unsuitable for the survival of larvae in pasture. This was due to very wide variations between minimum and maximum air temperatures and/or lack of sufficient moisture. The Molo area, from which the Romneys were obtained appears to be one of these unsuitable areas on account of temperature variations. This means that these animals may have had very little exposure while at the ADC farm. More importantly, the regular anthelmintic treatment that is routine on this farm may have reduced exposure and hence the opportunity to acquire resistance.

The cumulative egg counts illustrate how the breeds contaminated pasture at different levels. Contamination by the Red Maasai sheep was at a much lower level than that by the other three breeds. This means that the potential level of infection in young lambs would be much lower in pastures grazed by the Red Maasai alone compared to pastures grazed by any of the other breeds. Gibson

and Everett (1977) compared infective larval populations on paddocks grazed by sheep of the same age which were either resistant to *O. circumcincta* by exposure or were naive. Larval populations developing on the paddock grazed by the resistant sheep were less than a third of those in the paddock grazed by susceptible sheep. Six month old lambs which were subsequently grazed on the latter paddock acquired worm burdens up to 2.3 times higher than those found in lambs of the same age grazed on the first paddock.

The changes in mean PCV reflected the relative susceptibility of the four breeds; the PCV in the Red Maasai was the least depressed and that of the Romneys, the most depressed. PCV changes in the Dorpers and the Blackhead Somali were intermediate. The apparent improvement in PCV levels of the Romneys towards the end of the grazing period was due to the death of the most susceptible animals in the group and possibly also to the reduced stocking rates.

An interesting feature of this study was the similar pattern of egg counts and PCVs observed with the Dorper and the Blackhead Somali sheep. Similar egg counts of these two breeds are also evident in the study by Preston and Allonby (1979a) and may reflect their genetic relationship. The relatively lower susceptibility of the Blackhead Somali compared with the Dorper is probably due to a longer period of natural selection within a *Haemonchus* endemic area.

Antibody levels were clearly delineated between exotic and indigenous breeds with the latter having the higher levels. However, despite similar IgG responses in the Red Maasai and the Blackhead Somali, the Red Maasai was more resistant. The similar pattern observed with faecal egg counts and PCVs between the Dorper and the Blackhead Somali was not observed with IgG levels in the ELISA. These findings indicate that whatever role IgG plays in resistance against helminths, other more important factors are involved. One possibility is a more efficient local immune response and it has been shown (Preston, Duncan, Allonby and Morrison cited by Preston and Allonby 1979a) that Red Maasai wethers had higher mucosal anti-*Haemonchus* larval IgA than Merinos. Lack of

appropriate conjugates precluded the assay of mucosal anti-*H. contortus* IgA at the time of this study.

The effects of infection on weight gain were very marked in the Romneys but the picture is complicated by the high mortality which occurred in this breed. These animals had a net weight loss while the Blackhead Somali had the highest percentage weight gain. Although the ages of the experimental animals were only known to be between 10 and 15 months it is likely that most of the Blackhead Somali wethers were at the lower end of this range and therefore on a steeper growth curve than the others.

Following the artificial infection the differences in faecal egg output were maintained. However, a proper comparison between the Romney Marsh and the other breeds could not be made because only three animals of this breed were available for infection. In this part of the study the Red Maasai again shed fewer eggs than the Dorper and the Blackhead Somali. Preston and Allonby (1978) infected adult Red Maasai, Corriedale, Merino and Hampshire Down sheep, fed either a high or a low protein diet with a single dose of 350 *H. contortus* larvae/kg body weight. The egg counts in the Red Maasai on the high protein diet did not exceed 800 epg during the 14 weeks of sampling. In contrast counts in the Hampshires went as high as 10,000 epg while counts in the Corriedale and the Merino were intermediate. On the low protein diet, the faecal egg counts of the four breeds were generally much higher than those of the animals on the high protein diet but the order of relative susceptibility was maintained. In this case counts in the Red Maasai reached 2000 epg and those of the Hampshires, 30,000 epg.

In the present study there was no significant difference between the worm counts of the three breeds at necropsy although the Red Maasai had lower counts than the Dorpers and the Blackhead Somali. However, analysis of variance showed that the Red Maasai had significantly lower total burdens of adult *H. contortus* than the other breeds when counts in the animals which died during the

natural challenge period were included. It has been reported that the Red Maasai loses a large part of an established *H. contortus* worm burden between Days 15 and 25 after experimental infection and that this may be one reason for its increased resistance (Castellino, 1976). In the study by Preston and Allonby (1978) four breeds of sheep were experimentally infected with *H. contortus* and some lambs sacrificed four weeks later to assess the establishment while other lambs were faecal sampled until 14 weeks after infection. Worm counts in the Red Maasai supported Castellino's findings since they represented only 3.5% of the larval dose while comparative recoveries in the Merino, Corriedale and Hampshire Down were 13.0%, 15.2% and 22.8% respectively. Subsequent egg count differences reflected the earlier worm recoveries but no worm counts were available in the sheep sampled for the 14 weeks. Since there were no significant differences in the total worm counts at necropsy in the present study despite significant differences in egg counts, it would appear that another resistance mechanism may be directed at worm fecundity. In an earlier study in U.K., a group of sheep were trickle infected with *O. circumcincta* for 10 weeks, treated and then introduced into artificially contaminated pasture (Gibson and Everett, 1977). A second group of parasite-naive sheep were grazed in an adjacent similarly contaminated pasture. It was found that although both groups acquired similar worm burdens, the egg output of the group with prior exposure was much lower. This was attributed to inhibition of ovulation in adult female worms. In another experiment in U.S.A. (Yazwinski *et al.*, 1979), eight month old Dorset and Dorset x Barbados Blackbelly lambs were experimentally infected with a mixture of *Cooperia, Trichostrongylus, Ostertagia, Haemonchus* and *Oesophagostomum* larvae. Although there were no significant breed differences in total worm counts, the eggs produced per female worm in the case of *Cooperia, Ostertagia* and *Trichostrongylus* were significantly higher in the Dorset lambs: differences in egg production of *Haemonchus* and *Oesophagostomum* spp. were significantly different only in ewe lambs.

Similarly, although there were no differences in worm burdens in Florida Native and Dorset x Rambouillet sheep experimentally infected with *H. contortus*, Florida Native sheep shed significantly fewer eggs in faeces (Zajac *et al.*, 1990). Although there were no differences in worm counts at necropsy in the present study, there were highly significant differences in PCVs between the breeds. This may reflect differences in the hosts' haemopoietic capacities in infection (Whitlock, 1955a; Dargie and Allonby, 1975). Alternatively or in addition, there may have been a greater initial worm establishment in the Dorpers and the Blackhead Somali, but some of these worms were subsequently lost.

In conclusion, the results of this experiment showed that on the basis of egg count, PCV and mortality rate the Red Maasai was more resistant to naturally acquired *H. contortus* infection than the Dorper, Blackhead Somali and Romney Marsh. The order of increasing susceptibility was Red Maasai, Blackhead Somali, Dorper and Romney Marsh. The Romney Marsh was so susceptible that nine out of 15 animals died and productivity, as assessed by weight gains was most severely affected in this breed. Although the percentage gain was highest in the Blackhead Somali, this may have been influenced by differences in age between the four breed groups at the start of the experiment. Following an experimental infection with 10,000 *H. contortus* L_3, the Red Maasai maintained a lower egg output and a higher PCV than the other breeds. However, total worm counts at necropsy were not significantly different between the breeds although the Red Maasai had the lowest counts. This suggests that part of the resistance mechanism shown by the breed in this experiment was directed against egg production by adult female *Haemonchus*.

CHAPTER FOUR

INDIVIDUAL AND BREED VARIATION IN RESPONSE TO ARTIFICIAL AND SUBSEQUENT NATURAL *HAEMONCHUS CONTORTUS* INFECTION IN DORPER AND RED MAASAI EWES

4.1 INTRODUCTION

Genetic variation in the resistance of sheep to gastrointestinal parasites is well documented (Piper, 1987) and there is evidence for both within breed (Whitlock, 1955b) and between breed variation (Loggins *et al.*, 1965; Bradley *et al.*, 1973; Yazwinski *et al.*, 1979; Altaif and Dargie, 1978a, b). In Chapter 3, between breed variations in response to natural and subsequent experimental *H. contortus* infections were studied in male sheep of four breeds namely Red Maasai, Dorper and Blackhead Somali. In this chapter results of both between and within breed variations in response to *H. contortus* infections are presented. Young female Dorper and Red Maasai ewes were used and were given an artificial infection followed by exposure to natural infection on contaminated grazing. The ewes were also mated and they lambed during the study, which provided an opportunity to observe any breed differences during the periparturient period.

4.2 MATERIALS AND METHODS

4.2.1 Animals

Fifteen maiden Dorper ewes about 14 months of age were purchased from Manzoni Ranch in Machakos district. At the same time 13 Red Maasai ewes of about the same age were bought from ADC Mutara Ranch in Laikipia district. Another 15 Red Maasai ewes were purchased from the KARI substation of Transmara in Narok district.

All the animals were brought to NVRC Muguga, quarantined for two weeks and treated with fenbendazole (Panacur^{R}) at the rate of 10mg/kg body weight. The animals were housed together and the pen was cleaned twice a week to

exclude extraneous nematode infections.

4.2.2 Experimental design

A week after anthelmintic treatment each animal received a dose of 5000 *H. contortus* L3 orally. Thereafter faecal and EDTA blood samples were taken twice weekly for nine weeks; serum samples were obtained weekly. At nine weeks post infection the animals were treated with fenbendazole again and retained indoors for a month before being turned out in late August to graze together on an area known to be contaminated with *H. contortus*. The animals were housed at night and sampling continued as before.

After eight and half months of grazing, the ewes were put indoors for tupping and released six weeks later. During the tupping period the ewes were allowed to graze for two days a week.

Anti- *H. contortus* L3 antigen IgG antibodies were assayed in sera nine times before the tupping period using an ELISA. Subsequently, assays of anti- *H. contortus* adult worm IgG and total serum protein concentrations were carried out on ten and eight occasions respectively, while peripheral eosinophil levels were estimated weekly.

4.2.3 Feeding

While indoors the sheep were fed on commercial pelleted concentrates and hay. Water was provided *ad libitum.* The concentrates were also given as a supplement during the outdoor period.

4.2.4 Methods

All the parasitological, haematological, serological, biochemical and statistical methods used in this study are described in Chapter 2.

4.3 RESULTS

4.3.1 Experimental indoor challenge

Mortalities

One Dorper and three Red Maasai died of causes other than haemonchosis during this part of the experiment.

Faecal egg counts

The changes in mean faecal nematode egg output are shown in Figure 4.1a. In the Dorpers, egg counts rose over a period of three weeks to a peak of 3900 epg on Day 42. After a temporary decline to 2300 epg on Day 49, the egg count rose again to reach a higher peak of 9600 epg one week later. At the end of the experiment the mean egg count of the Dorpers was 8500 epg. In the Red Maasai, the egg count increased gradually to around 2000 epg by seven weeks post infection; they then showed a sharp rise to a peak of 5900 epg on Day 59. The mean egg output remained lower than that of the Dorpers throughout the nine weeks except on one occasion (Day 59). The mean egg output of the Dorpers during the sampling period was almost twice the mean of the Red Maasai and the difference between the two breeds from patency was highly significant (Table 4.1). Individual animal variation was highly significant but breed by day interaction was not. Three worm-free wethers kept together with the ewes as extraneous infection controls had zero egg counts during the experiment.

PCVs

Changes in the mean PCVs are shown in Figure 4.1b. In both breeds the PCV fell from 35% at the beginning of the experiment to 19% on Day 56. From then on the PCVs appeared to be rising. There was no significant breed difference in PCVs but individual animal variation was highly significant as were the day to day variations (Table 4.1). The breed by day interaction was not significant.

Tabie 4.1: Results of analysis of variance of faecal egg counts and PCVs of Dorper and Red Maasai ewes infected with a single oral dose of 5000 *H. contortus* L_3

Source	Probability	
	$Log_{10}EPG$	PCV
Breed	0.0017	0.7761
Animal(breed)	0.0001	0.0001
Day	0.0001	0.0001
Breed*day	0.1507	0.7349

Model: Parameter = breed animal(breed) day breed*day. Parentheses indicate animal nested within breed while * indicates breed by day interaction

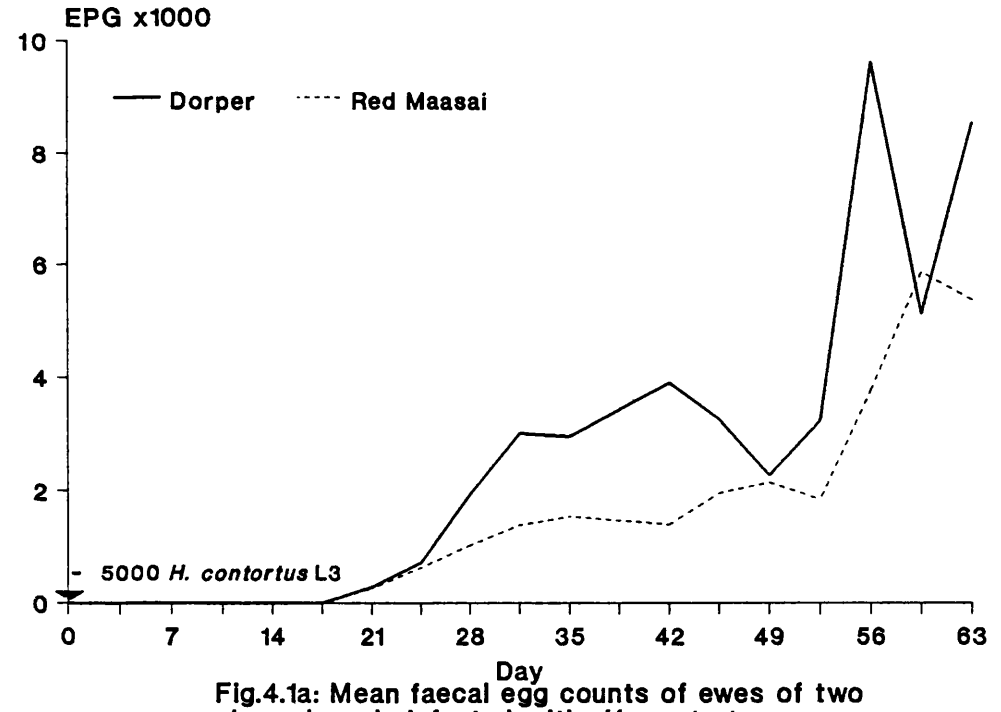

Fig.4.1a: Mean faecal egg counts of ewes of two sheep breeds infected with *H. contortus*

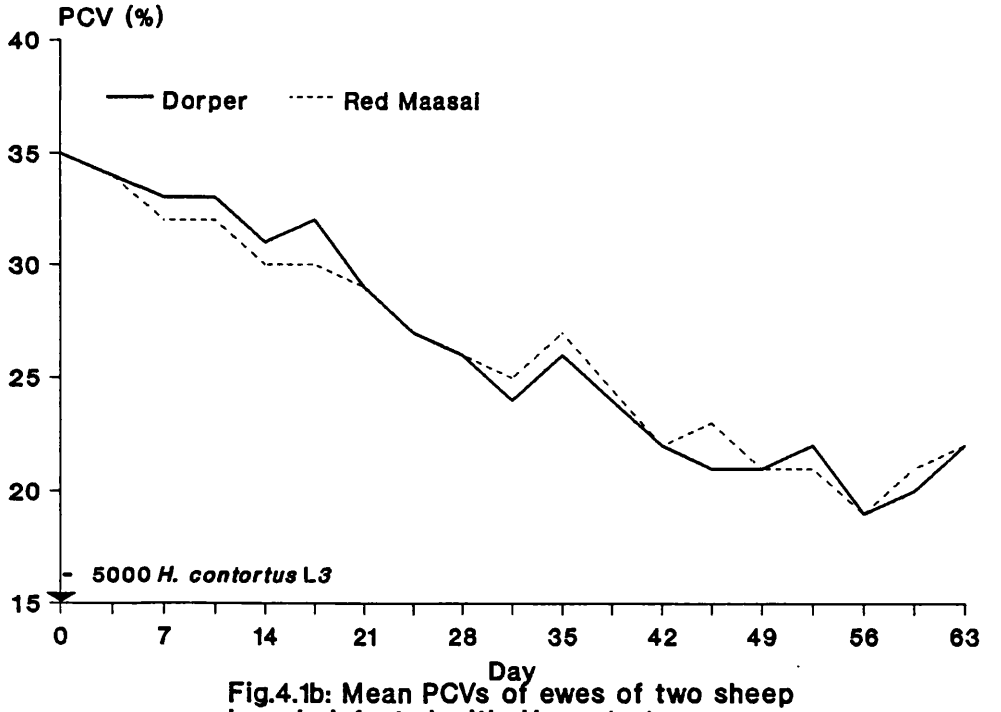

Fig.4.1b: Mean PCVs of ewes of two sheep breeds infected with *H. contortus*

4.3.2 Natural field challenge

Mortality

One Red Maasai ewe which died of peritonitis during this part of the experiment had a negligible worm burden which included 20 *H. contortus*.

Faecal egg counts

The Red Maasai ewes generally shed fewer eggs than the Dorpers (Fig.4.2a). Average egg counts rose rapidly from zero (Septe..ber values not shown) to peak in late October 1991 in the Dorpers and the Red Maasai at 5300 and 2700 epg respectively. Over the following six and half months after which the ewes were put indoors for tupping, the egg counts fluctuated between 500 and 3600 epg in the Dorper and 500 to 2000 epg in the Red Maasai.

From tupping through to lambing, egg counts fluctuated between 500 and 4400 in the Dorpers, the peak occurring in July 1992. About two weeks to lambing a definite and sustained rise was observed and ultimately a peak of 11,350 epg was attained in early November. Egg counts in the Red Maasai varied between 500 and 2000 epg until the beginning of September; they then rose to fluctuate between 2000 and 4000 epg. As in the Dorper the Red Maasai attained the highest egg count in early November, when a mean of 5300 epg was recorded. From the average egg output in the two breeds over this period of increased egg output the Dorpers were shedding about twice the number of eggs shed by the Red Maasai (Table 4.2). Faecal egg counts carried out in December, five weeks after the end of the experiment and approximately three weeks after the end of the lambing period showed a substantial decline in egg output in the Dorpers but not in the Red Maasai (Fig. 4.2a). Cumulative mean egg counts showed that the Dorpers had potential to contaminate the pastures twice as much as the Red Maasai (Fig.4.2b).

On statistical analyses there was a highly significant ($p<0.008$) breed difference in mean faecal egg counts with the Dorpers having the higher counts (Table 4.3).

Individual animal variation and daily variations were highly significant ($p<0.0001$). There was no significant breed by day interaction.

PCVs

The PCVs fluctuated markedly but in general showed an upward trend with time starting at below 25% in late August 1991 to over 30% in both breeds just before tupping (Fig 4.3). Initially the Red Maasai had lower mean PCVs than the Dorpers but from November 1991 the PCVs in the Red Maasai were generally higher.

From tupping the mean PCV gradually fell to 25% in the Dorper and to 24% in the Red Maasai in early November 1992.

There was no significant breed difference in PCVs but there was a highly significant breed by day interaction and individual animal and daily variations were also highly significant (Table 4.3).

ELISA O.D.s

Serum anti-*H. contortus* L_3 antigen antibodies increased to a peak in August 1992 and started declining thereafter (Fig.4.4). The Red Maasai ewes generally showed higher responses than the Dorpers.

There was no significant overall breed difference in O.D. values between the Red Maasai and the Dorper, with adult worm antigen (Table 4.4) and the changes followed a similar pattern to the changes with L_3 antigen (Fig. 4.4). However, individual variation and daily changes were highly significant. The breed by day interaction was also significant ($p<0.004$). With larval antigen only the individual and daily variations were significant ($p<0.0001$).

Peripheral eosinophil counts

Mean eosinophil counts fluctuated in the Red Maasai but generally fell with time while those of the Dorpers also fluctuated but showed several increases

(Fig.4.5). The lowest values attained in both breeds occurred in the last four weeks prior to lambing. The Red Maasai generally had higher values than the Dorpers except in the last few weeks of the experiment when the Dorpers had higher values.

There was no significant breed difference in eosinophil counts but there was a significant breed by day interaction and individual animal and daily variations were also highly significant (Table 4.3).

Total serum protein

The mean total serum protein (S.D.) levels were 62.6 (7.1) and 63.9 (4.5) g/l for Dorpers and Red Maasai respectively and the differences were not significant ($p<0.33$). However, daily and individual animal variations were highly significant (Table 4.4).

4.3.3 Within breed differences

Within breed differences in egg output, PCVs and eosinophil counts were also observed. As examples, Figure 4.6a illustrates differences in egg counts between the most 'susceptible' and the most 'resistant' of the Red Maasai and Dorpers. Their PCVs are also shown (Fig. 4.6b). These pairs of animals were chosen on the basis of egg counts during the periods of experimental challenge and natural challenge from tupping. The Dorpers showed more marked differences than the Red Maasai in terms of faecal egg counts and PCVs, while differences in other parameters were less marked (Table 4.5) and there were some inconsistencies within the breeds. For example, the 'resistant' Red Maasai had a consistently lower PCV than the 'susceptible' Red Maasai. Similarly, when the most resistant Dorper no. 450 was compared with another Dorper with a medium egg count, the latter had noticeable higher PCV, eosinophil counts and total protein levels (Table 4.6).

Table 4.2: Mean (± S.D.) faecal egg counts, PCVs and eosinophil counts before and during the periparturient period of Dorper and Red Maasai ewes exposed to natural *H. contortus* infection.

Breed	Egg counts (epg)	PCV (%)	Eosinophil count (x10^6 cells/ml)
Entire period from tupping			
Dorper	3612[a] (4702)	27.4[a] (4.0)	0.57[a] (0.58)
Red Maasai	2067[b] (2415)	28.5[b] (3.4)	0.74[b] (0.67)
Tupping to three weeks before lambing			
Dorper	2450[a] (3652)	28.0[a] (4.0)	0.56[a] (0.42)
Red Maasai	1311[b] (1677)	29.6[b] (3.2)	0.85[b] (0.71)
Periparturient period			
Dorper	6048[a] (5702)	25.9 (3.5)	0.60 (0.85)
Red Maasai	3377[b] (2991)	26.4 (2.9)	0.51 (0.50)

Different superscripts indicate a significant difference ($p<0.001$). No superscript indicates there is no difference

Table 4.3: Results of analysis of variance of faecal egg counts, PCVs and eosinophil counts of Dorper and Red Maasai ewes naturally infected with *H. contortus.*

Source	Probability		
	$Log_{10}EPG$	PCV	$Log_{10}Eosinophils$
Breed	0.0077	0.1091	0.1554
Animal(breed)	0.0001	0.0001	0.0001
Day	0.0001	0.0001	0.0001
Breed*day	0.3268	0.0015	0.0489

Model: Parameter = breed animal(breed) day breed*day

Table 4.4: Results of analysis of variance of ELISA O.D. values and total serum proteins of Dorper and Red Maasai ewes naturally infected with *H. contortus*

Source	Probability		
	Log_{10}ELISA O.D		Total serum protein
	Adult antigen	L_3 antigen	
Breed	0.3404	0.2498	0.3291
Animal(breed)	0.0001	0.0001	0.0001
Day	0.0001	0.0001	0.0001
Breed*day	0.0035	0.1082	0.3133

Model: Parameter = breed animal(breed) day breed*day

Table 4.5: Egg counts, PCVs, eosinophil (Eos.) counts, O.D.s with both L_3 and adult worm antigen and total protein (TP) levels (±S.D.) of a pair of 'resistant' and 'susceptible' Dorper and Red Maasai ewes from tupping to lambing in a natural *H. contortus* exposure.

	Dorpers			Red Maasai		
	no. 401		no. 450	no. 364		no. 2031
EPG	12084(7176)	*	982(1036)	796(1510)	*	3758(2734)
PCV	21.10(5.20)	*	27.10(4.20)	26.60(2.60)	*	29.20(2.50)
Eos.	0.14(0.10)	*	0.34(0.21)	0.78(0.35)		0.95(0.67)
O.D.[1]	0.19(0.07)		0.22(0.06)	0.34(0.07)		0.27(0.08)
O.D.[2]	0.16(0.07)		0.19(0.09)	0.27(0.07)		0.23(0.08)
TP	57.40(7.20)		58.30(4.20)	64.00(2.20)		65.00(2.20)

* Denotes obvious differences between the pair in each breed. [1]O.D.s with larval and [2]with adult worm antigens

Table 4.6: Egg output, PCVs, eosinophil counts, O.D.s with both L_3 and adult worm antigen and total protein (TP) levels (S.D.) of a relatively 'resistant' (no. 402) and a 'resistant' (no. 450) Dorper ewe exposed to natural *H. contortus* infection.

Parameter	no. 450		no. 402
EPG	982(7176)	*	3017(3859)
PCV	27.10(4.20)		28.90(2.50)
Eosinophils	0.34(0.10)	*	0.69(0.72)
O.D.[1]	0.22(0.06)	*	0.31(0.06)
O.D.[2]	0.19(0.09)	*	0.27(0.07)
TP	58.40(4.20)	*	68.00(6.80)

* Denotes obvious differences between the pair. [1]O.D.s with larval and [2]with adult worm antigens

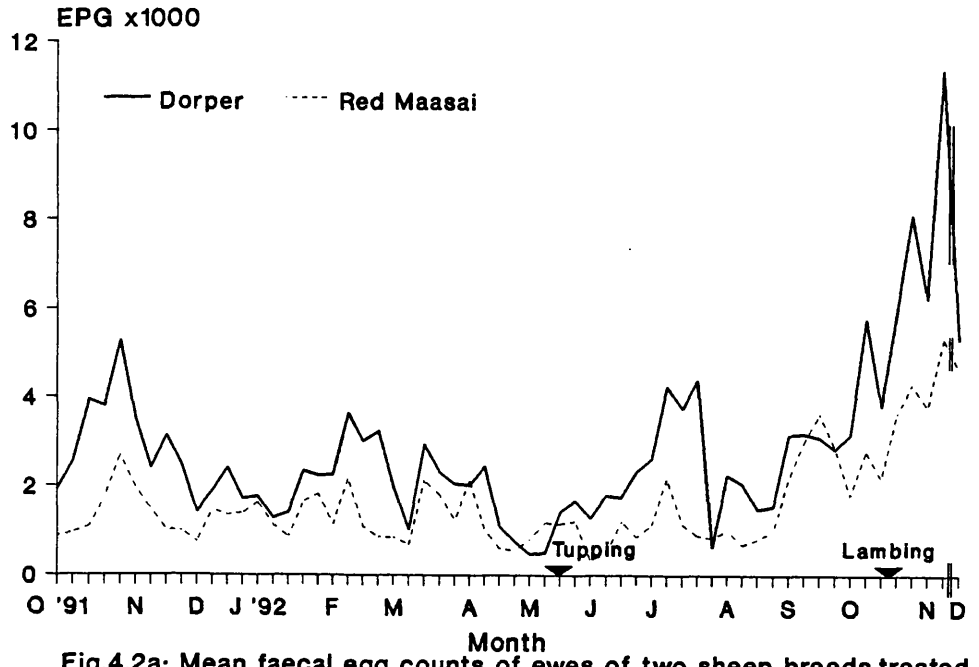

Fig.4.2a: Mean faecal egg counts of ewes of two sheep breeds treated after artificial *H. contortus* infection and re-infected naturally

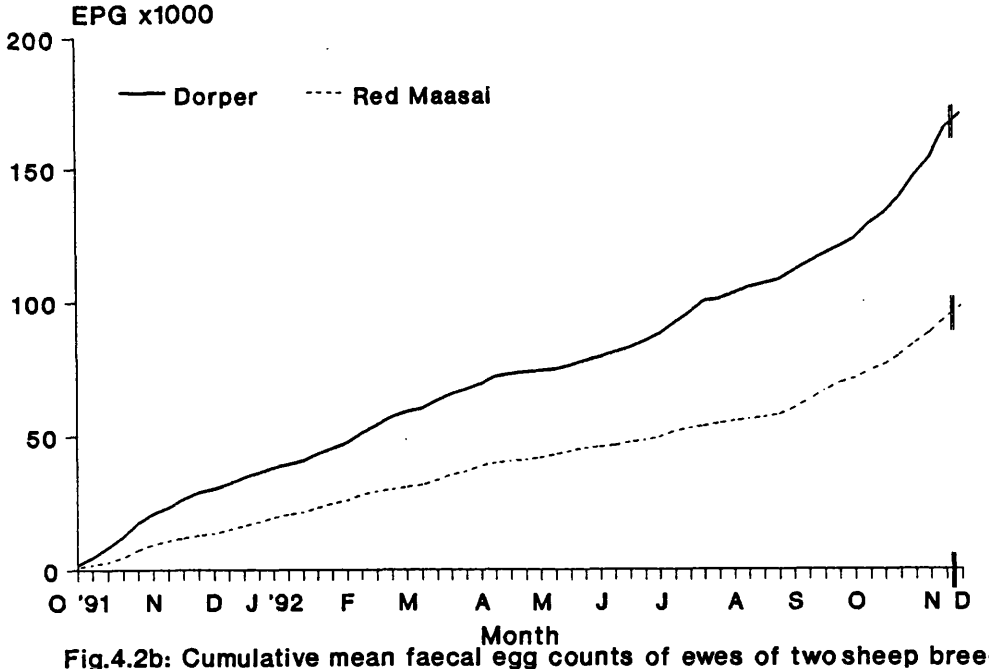

Fig.4.2b: Cumulative mean faecal egg counts of ewes of two sheep breeds treated after artificial *H. contortus* infection and re-infected naturally

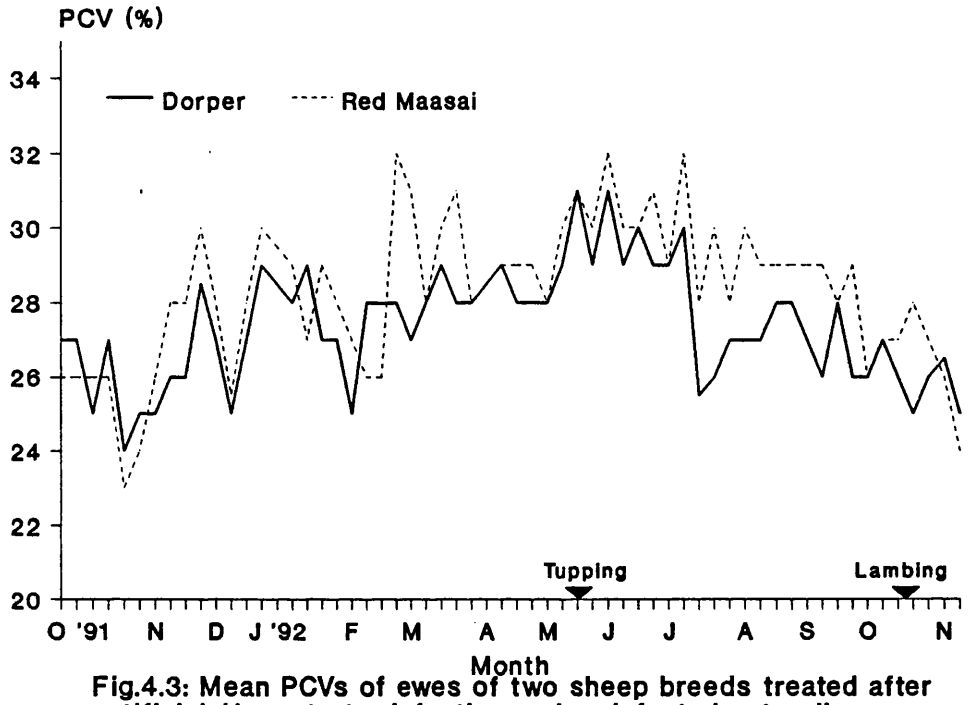

Fig.4.3: Mean PCVs of ewes of two sheep breeds treated after artificial *H. contortus* infection and re-infected naturally

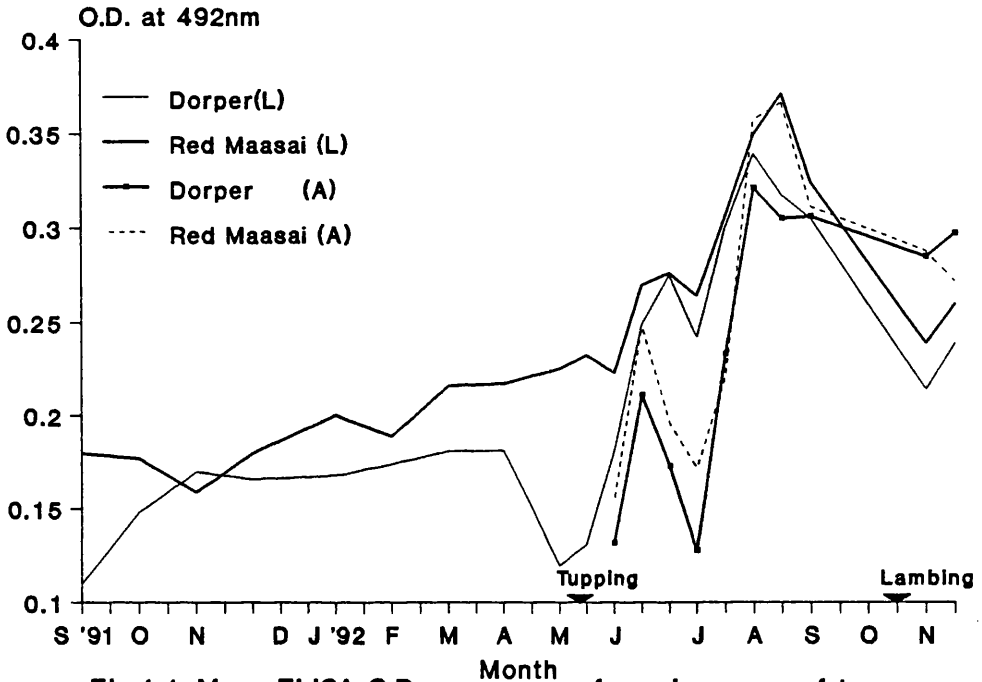

Fig.4.4: Mean ELISA O.D. responses of grazing ewes of two sheep breeds to larval (L) and adult (A) *H. contortus* antigens

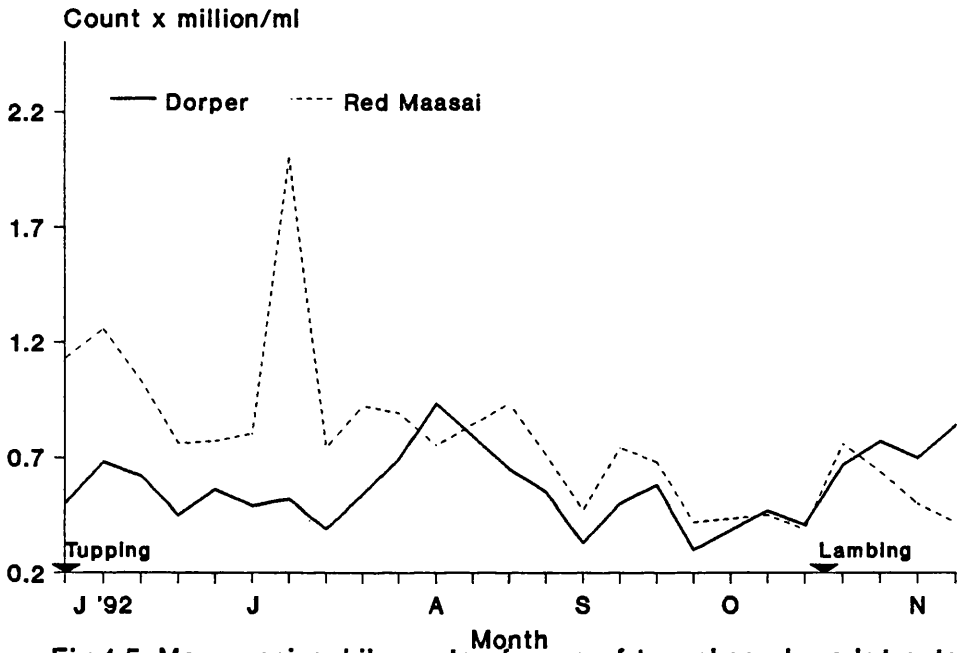

Fig.4.5: Mean eosinophil counts of ewes of two sheep breeds treated after artificial *H. contortus* infection and re-infected naturally

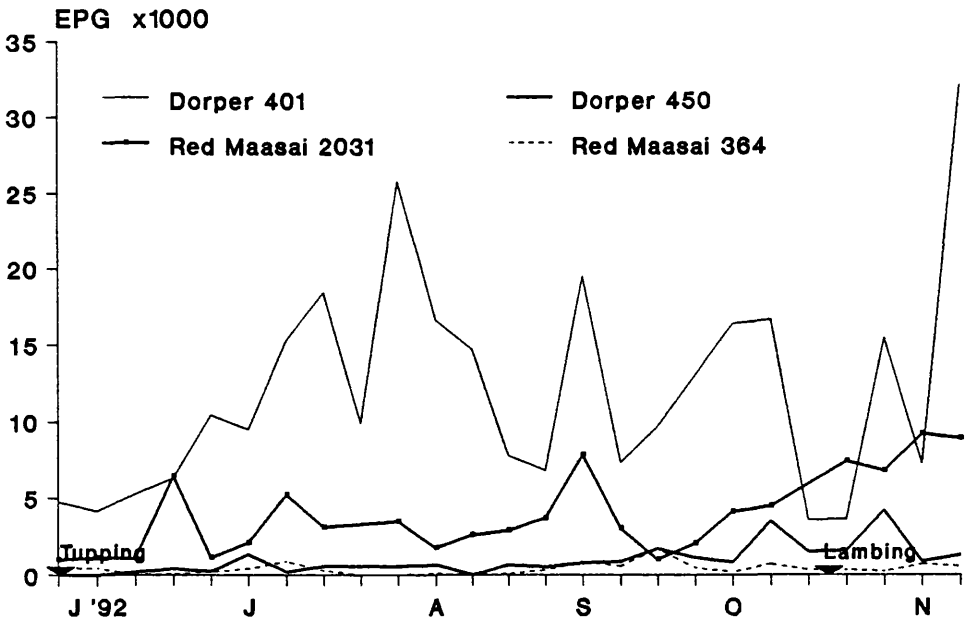

Fig.4.6a: Faecal egg counts of 'susceptible'(401,2031) and 'resistant' (450,364) Dorper and Red Maasai ewes naturally infected with *H. contortus*

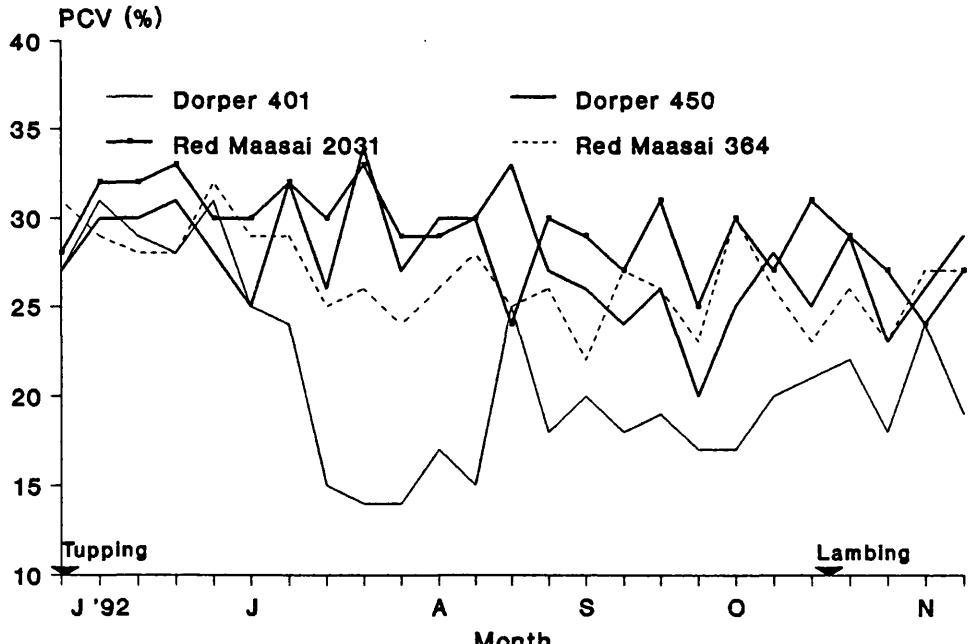

Fig.4.6b: PCVs of 'susceptible'(401, 2031) and 'resistant' (450,364) Dorper and Red Maasai ewes naturally infected with *H. contortus*

4.4 DISCUSSION

On the basis of egg count data there were clear differences between the Red Maasai and Dorper ewes after either artificial or natural *H. contortus* infection. There were no significant breed by time interactions, implying that the differences were consistent throughout the study. Total worm count data were not obtained because the ewes were kept for breeding.

During the artificial infection period, egg output was significantly different between the breeds with the Red Maasai having lower counts. This difference could have been due to differences in worm establishment, fecundity, worm expulsion or differences in the female to male ratio. Since PCVs fell in the same way in both breeds it is more likely that the egg count differences reflected differences in host effects on the established worms rather than on the numbers established. Indeed, the results of the experiment described in Chapter 3 showed that after experimental infection the Red Maasai continued to shed significantly fewer eggs than the Dorper and the Blackhead Somali although there were no significant differences in total worm counts at necropsy. Since earlier work showed that the Red Maasai lost a substantial part of an established worm burden 15-25 Days after artificial infection (Castellino, 1976), the findings in our two experiments suggest that the Red Maasai could be expressing resistance in more than one of the ways mentioned above. The results of this study are similar to those reported by Zajac *et al.* (1990). In their study, nine to 10 month old St. Croix, Florida Native and Dorset/Rambouillet sheep infected with 20,000 *H. contortus* larvae had similar total worm burdens at necropsy. Despite this, the Dorset/Rambouillet sheep showed much higher faecal egg counts than the other two breeds (peak counts of 26,500 in this breed against 5325 and 430 epg in Florida Native and St. Croix respectively). Similarly, Barbados Blackbelly x Dorset lambs shed significantly fewer eggs of *Cooperia, Trichostrongylus* and *Ostertagia* spp. than Dorset lambs although the worm burdens were similar (Yazwinski *et al.*, 1979). This was found to be due to differences in fecundity of

the two worm populations.

It has been shown previously that the response to primary infection alone may fail to show clear differences between breeds (Courtney *et al.*, 1985a; Gamble and Zajac 1992) or between resistant and random bred lines (Gill, 1991). In the present experiment breed differences were observed after a single experimental infection although this was not a true primary infection since both breeds had previous natural exposure. There could also have been an age effect since it has been shown that 15 week old parasite-naive St.Croix lambs produced fewer eggs than Dorsets after a primary infection while there was no such difference with eight week old lambs of the same breeds (Gamble and Zajac, 1992).

No obvious differences in mean PCVs between the two breeds were observed in this study. Dargie and Allonby (1975) found that the most marked haematological disturbances in sheep given single doses of 10,000 *H. contortus* L_3 occurred between Days 12 and 25 post infection. During this time PCVs in infected sheep fell from an average of 33% to 22%. Thereafter the values either increased steadily or were maintained at a reduced level. In the present experiment the decline in PCVs occurred over a longer period, that is, up to Day 42. The lack of any apparent differences in PCVs does not necessarily mean that blood loss was similar in both breeds. This is because the anaemia, as indicated by PCV determination at any point in the course of an infection largely depends on the host's erythropoietic potential. Hence it may fail to accurately reflect the amount of blood being lost through haemorrhage (Dargie and Allonby, 1975). However, the fact that there was no significant ($p< 0.73$) breed by day interaction suggests that the changes in PCVs were occurring in the same way in both breeds. Lack of a significant difference in PCVs between groups of sheep after natural infection despite significant differences in faecal nematode egg output has been noted between Merino sheep selected for increased susceptibility to *H. contortus* and a random-bred line (Woolaston *et al.*, 1990). In contrast, after a prior artificial infection the same two groups of sheep

showed significant differences in PCVs but not in egg counts. A third line selected for increased resistance maintained significantly higher PCVs and lower egg counts than both the susceptible and the random bred lines after either artificial or natural infection.

Following natural infection in this study, the significant breed differences in faecal egg output observed after the artificial infection continued to be evident. The pattern of egg count changes also occurred in a similar manner in the two breeds as implied by the non-significant ($p< 0.15$) breed by day interaction. Egg counts peaked later and remained at a lower level than those observed following the artificial infection. This could be due in part to a degree of acquired resistance following the artificial infection. It could also be due partly to continuous intake of larvae from pasture since it has been shown that in trickle infections, egg counts peak earlier and at a lower level than in single infections (Dineen *et al.*, 1965). More dramatic breed differences in natural *H. contortus* infections have been reported with St. Croix and Dorset lambs (Gamble and Zajac, 1992). When lambs of the two breeds were grazed together on contaminated pasture, Dorset lambs shed significantly greater numbers of eggs than St. Croix from Day 47 after exposure until treatment on Day 108. The mean faecal egg counts were 3822 epg and 218 epg for Dorsets and St Croix respectively. Following drug treatment, lambs of both breeds became re-infected but to a much lower level than before; St Croix shed a mean of only 10 epg while the Dorsets shed an average of 693 epg.

Cumulative mean egg counts illustrated the relative potential contribution to pasture contamination of the two breeds at any sampling point. Assuming that the average faecal egg output was the same, the 14 Dorpers had the potential to contaminate the pastures at almost twice the level of the 17 Red Maasai.

Towards the end of the experiment a periparturient rise (PPR) in egg output was observed in both breeds. From the average egg output in the two breeds over the PPR it appeared that the level of pasture contamination by the Dorpers

remained at twice that of the Red Maasai but there were no breed differences in eosinophil counts and in PCVs during this period. In a number of American studies it has been shown that while non-lactating domestic, exotic and cross-bred ewes showed no significant breed differences in naturally acquired *H. contortus* worm burdens (Courtney *et al.*, 1985b) significant differences were seen in lambing ewes (Courtney *et al.*, 1984). This suggested that the observed differences were due to breed effects on the relaxation in immunity during the PPR. Woolaston (1992) compared peri-parturient worm egg counts in 395 Merino ewes bred for either increased or decreased resistance to *H. contortus*; an unselected control group was included. In all of the sheep a rise in egg counts began four weeks before parturition and continued into lactation. Although all three lines showed this PPR in egg counts, the counts were significantly lower at all stages in the sheep bred for increased resistance than in the susceptible line; counts in the control group were intermediate. These results suggested that there was less contamination of pasture from the resistant than from the susceptible line of sheep both before and during the PPR. They also suggested that pasture contamination would be higher when the susceptible line was grazed alone than when the two lines were grazed together. This suggestion is perhaps relevant to the findings of the present study. Similar results were obtained by Courtney *et al.* (1986) who found significant ($p<0.05$) differences in the magnitude of the PPR in faecal egg counts between three strains of Florida Native sheep. Lactating 'University' strain ewes which had been selected over a 26 year period for parasite resistance had lower faecal egg counts than ewes of two other strains which had been treated regularly with anthelmintics for prolonged periods of time. In a between breed study similar to that of Courtney *et al.* (1984), *H. contortus* resistant Florida Native ewes were compared with susceptible Dorset/Rambouillet ewes during a peri-parturient period (Zajac *et al.*, 1988). The PPR occurred in the Dorset/Rambouillet ewes from one week before the average lambing date to six weeks post-lambing. These ewes attained a peak egg output

of 1760 epg compared with 940 epg in unbred ewes of the same breed. In addition the lactating ewes had fewer arrested larvae and more adults than the unbred ewes at necropsy. In contrast the Florida Native ewes had consistently low egg counts throughout the period with a peak of only 300 epg and no differences were observed between lactating and unbred ewes of this breed. However, although the rise in faecal egg output was not apparent in the Florida Native ewes, there was some immunosuppression in that at necropsy lactating animals had a lower proportion of arrested larvae and larger total worm burdens than the unbred ewes.

PCVs rose slowly over several months after the ewes were turned out to pasture. This could be further evidence that some resistance developed following the artificial infection and was now being manifested after natural challenge. It is also the case that the faecal egg counts at pasture contained eggs from several species of gastrointestinal nematodes which do not usually depress the PCV. These accounted for about 10-25% of the larvae in faecal cultures of faeces from the ewes and consisted mainly of *Oesophagostomum* and *Trichostrongylus* spp. The gradual fall in PCVs seen during the tupping period may be related to housing and diet and the continued fall towards the end of the study could be linked to increased susceptibility to *Haemonchus* infection during the periparturient relaxation in immunity.

There were no significant ($p< 0.11$) differences between the breeds in PCVs. However, the highly significant ($p<0.002$) breed by day interaction indicated that at certain times the PCVs were significantly different and this occurred mostly in favour of the Red Maasai.

Overall peripheral eosinophil responses were not significantly different between the two breeds but the significant ($p<0.05$) breed by day interaction indicated that differences were significant at certain times. The blood eosinophil response in mammals has two main features; its T-lymphocyte dependence and its propensity to occur during parasitic infestations (Sewell and Vadas, 1983).

The role of eosinophils in resistance is yet to be fully elucidated, but it has been shown in *Trichostrongylus colubriformis* infections that the numbers of circulating eosinophils reflect immunological responsiveness rather than the degree of parasitism (Dawkins *et al.*, 1989; Windon, 1991). The greater numbers of circulating and small intestinal eosinophils found in a *T. colubriformis* resistant line compared with a susceptible line of guinea pigs were thought to be responsible for differences in susceptibility shown by the two lines to this parasite (Handlinger and Rothwell, 1981). In *H. contortus* infections it has been suggested that eosinophils make the gut unfavourable to larval establishment (Hunter and Mackenzie, 1982). However, although significant increases in peripheral eosinophil responses were observed in Merino lambs of resistant and susceptible lines after both primary and secondary infections, the levels of eosinophilia were not significantly different between the lines (Gill, 1991). In fact the resistant line showed a lower response than the susceptible line after the second infection. In contrast, three immunising infections with 10,000 *H. contortus* L3 did not cause significant increases in pre-challenge eosinophil counts in immune sheep following challenge infection (Adams, 1993).

In this study, there were no significant breed differences in serum anti-*H. contortus* L3 IgG responses. With adult worm antigen, there was a significant breed by day interaction which implied that significant differences occurred at certain times; in general the Red Maasai had higher levels at such times. Using an ELISA, Gamble and Zajac (1992) noted occasional significant differences in serum IgG levels to L3, exsheathing fluid or second moult cuticle antigens in St. Croix and Dorset lambs after natural *H. contortus* infection. On such occasions the more resistant St Croix had higher values than the Dorsets. Sera from infected groups of both breeds contained significantly elevated levels of parasite-specific antibodies compared with pre-exposure sera. Gill (1991) found significantly ($p<0.04$) higher antibody responses to adult worm antigen in resistant than in susceptible Merino lambs on Day 35 after a second *H. contortus*

infection. Resistant lambs also displayed consistently higher anti-larval antibody responses although the differences between the groups were not significant.

In general, serum antibody responses do not appear to differ between breeds or between resistant and susceptible strains of sheep after infection (or vaccination) although there may be marked parasitological differences (Zajac *et al.*, 1990). However, in one vaccination trial in two to three month old lambs with irradiated *T. colubriformis* L3, complement fixing antibodies proved to be the most useful immunological parameter for correlation with resistance based on faecal egg counts (Windon and Dineen, 1981). The disparities frequently encountered between serological and parasitological data could be due, at least in part, to the use of crude somatic antigens which are poor sources of 'functional' antigens (Silverman, 1965). A less crude extract is likely to elicit serum antibody responses which will correlate significantly with protection. Thus, an intestinal membrane protein H11, from adult *H. contortus* afforded 92-95% protection in terms of reduction in egg output in vaccinated compared with unvaccinated sheep and the level of protection positively correlated with serum antibody titres to H11 (Munn *et al.*, 1993; Smith *et al.*, 1993). In some cases, however, the serum responses to H11 have failed to correlate with protection since it is a relatively large glycoprotein with perhaps only a few protection-relevant antigenic epitopes (Smith and Smith, 1993). In the present study, the Red Maasai appeared to mount a better serum IgG response against adult *H. contortus* antigens than the Dorper. The differences were significant only at certain times as implied by the significant ($p< 0.004$) breed by time interaction. The relationship between this response and the greater resistance shown by the Red Maasai in terms of faecal egg output is not known, but in this context, Gill (1991) found that in resistant Merino lambs, antibody levels against antigens of adult *H. contortus* from patency to termination of the experiment were negatively correlated ($r= -0.4$ to -0.5) with the worm burdens.

Serum albumin and total proteins are usually reduced in helminth infections

(Steel *et al.*, 1982; Holmes, 1987). In the present study, total protein concentrations were lower, though not significantly so, in the Dorper compared with the Red Maasai. It has been shown that the breed of animal and dietary protein levels may influence serum protein concentrations in helminth infection. For example, while there was little effect on total protein, albumin and globulin levels in Scottish Blackface lambs infected with 125 *H. contortus* L3/kg body weight on a high protein diet, all three parameters showed a gradual fall in infected lambs on a low protein diet (Abbott *et al.*, 1985a). In contrast, marked reductions in serum protein levels were observed in similarly infected Finn Dorset lambs on both high and low protein diets. No changes were seen in uninfected lambs of either breed fed on either of the protein rations. It was also found that sheep infected with 200 *Fasciola hepatica* metacercariae and fed on a ration containing 6% protein experienced more rapid hypoalbuminaemia than similarly infected sheep on a 13% protein diet (Berry and Dargie, 1976).

In this study considerable within breed variation in the parameters measured occurred. For example, the resistant Red Maasai (no. 364) had significantly lower faecal egg counts than the susceptible animal (no. 2031) while the latter had higher PCVs. Perhaps it is pertinent to observe that sheep may be classified into two groups on the basis of their potential PCV changes in infection (Conway and Whitlock, 1965; Coadwell and Herd, 1975). These workers found that the propensity for PCVs to fall to dangerous levels following infection is higher in some sheep than in others. Furthermore, it was observed in this study that the Red Maasai ewes from Transmara on average had higher PCVs than those from Mutara, although the latter had on average, lower egg counts. Sheep no. 364 whose egg count was mostly below 1500 epg throughout the study was from Mutara while sheep no. 2031 was from Transmara. The implication of these observations needs clarification. The Dorpers also showed marked individual variations. Clunies Ross (1932) noted individual differences in experimentally infected Merino lambs; the differences were in respect of

resistance to infection and resistance to the effects of infection. In addition, within experimental groups of animals different self-cure phenomena have been shown to occur (Dargie and Allonby, 1975) and it should be noted that resistance based only on faecal egg counts may not always reflect true resistance.

In conclusion, the results of this experiment showed that Red Maasai ewes were more resistant than Dorper ewes after artificial and natural *H. contortus* infections on the basis of significant differences in faecal egg counts. The breed differences in PCVs, serum antibody levels, peripheral eosinophil responses and total serum proteins were less clear-cut. It is possible that the levels of infection were not sufficiently high for the differences to be more clearly expressed. The relaxation in immunity during the peri-parturient period appeared to occur with the same magnitude in both breeds.

Also in this study there were substantial within breed differences in resistance which appeared to be more marked in the Dorper than in the Red Maasai. The greater within breed differences in the Dorper probably suggests that natural selection has occurred to a lesser extent in this breed than in the Red Maasai which has evolved in a *Haemonchus* endemic area with little or no anthelmintic therapy (Preston and Allonby, 1978; Urquhart, 1988).

CHAPTER FIVE

RESPONSE OF DORPER AND RED MAASAI LAMBS TO TRICKLE *HAEMONCHUS CONTORTUS* INFECTIONS

5.1 INTRODUCTION

In the field, animals are constantly exposed to infective larvae and therefore single experimental infections do not necessarily bear a close resemblance tc the pattern of infection found under field conditions (Abbott *et al.*, 1988). It is impracticable to experimentally administer infective larvae to animals in a manner which closely simulates the natural situation but it is considered that administration of continuous, small doses can mimic the gradual build up of infection encountered naturally (Sykes and Coop, 1976). Various experiments have been carried out in which trickle infection regimens have been used. In some studies where responses to single and trickle infections with *H. contortus* have been compared (Andrews, 1942; Manton *et al.*, 1962; Dineen *et al.*, 1965) the single infection was found to be more pathogenic. Thus when two to four month old Dorset Horn lambs received 3000 L3 either in two equal doses 30 days apart or in doses of 100 larvae at two day intervals, a less severe anaemia occurred in the trickle infected lambs than in the lambs infected with two large doses (Manton *et al.*, 1962). Similar results were obtained with 10-12 month old Down lambs infected with a total of 9000 larvae using the above regimens of infection in the same experiment. The egg counts rose rapidly with single infections while the rise was more gradual and peaked at a lower level with trickle infections. Similar findings were reported with Merino x Border-Leicester lambs when they were infected with 3000 *H. contortus* L3 either in a single dose or in daily doses of 100 larvae for 30 days (Dineen *et al.*, 1965). In the latter study three of the 19 lambs given the single infection died of haemonchosis while no mortality occurred in the trickle infection group. In contrast, Abbott *et al.* (1988) obtained a 50% mortality in eight Finn Dorset lambs fed a low protein

diet and infected with 100 *H. contortus* L3/kg body weight followed by 200 larvae three times a week for 17 weeks. In eight similar lambs fed on the same low protein diet but exposed to a single dose of 350 L3/kg body weight, mortality was also about 50% (Abbott *et al.*, 1986). Although the biochemical and haematological changes in the trickle infected group developed more gradually relative to the single infection group, the ultimate severity was the same in both groups (Abbott *et al.*, 1988). However, the total dosage appeared to be higher in the trickle infected group when these dosages were calculated on the basis of average weights of the lambs at the start of the experiment. Thus each lamb in the single infection group received an average of 9000 L3 compared to 13,000 L3 in the trickle infection group. In another study, higher pathogenicity was found in lambs infected with small daily doses of larvae than in those given the same total number of larvae weekly, although no significant difference in faecal egg counts was observed (Pradhan and Johnstone, 1972a, b).

The present experiment was designed to investigate any differences in the responses of Dorper and Red Maasai sheep to experimental trickle *H. contortus* infections.

5.2 MATERIALS AND METHODS

5.2.1 Animals

Four to five month old male Dorper lambs were purchased from Sosian Ranch in Laikipia District. Red Maasai male lambs of the same age were purchased from pastoralists from both Laikipia and Samburu districts. The lambs were brought to N.V.R.C., treated with fenbendazole at 10mg/kg body weight and quarantined for three weeks. During the last week of quarantine the lambs were castrated with a burdizzo and kept for a further three weeks to allow recovery from the stress of castration. Thereafter the lambs were transported to the animal husbandry station of KARI, 3km away where the experiment was conducted.

5.2.2 Experimental design

The animals were randomly divided into four groups each of which was allocated its own pen. The two infected groups of Dorper and Red Maasai lambs had 17 animals each while the uninfected control groups had 15 Dorpers and 19 Red Maasai respectively.

Each of the lambs in the infection groups received 1000 *H. contortus* L_3 in Weeks 0, 1, 2, 3, 4, 5, 6, 8 and 10 of the experiment. Faecal egg counts and PCVs were determined once a week and eosinophil counts were determined on seven occasions during the last eight weeks of the experiment. Total serum proteins were assayed in Weeks 1, 9 and 13. The animals were weighed once a week and the experiment was terminated two months after the last infection.

The sheep pens were cleaned twice a week to minimise the risk of extraneous nematode infection.

5.2.3 Feeding

The lambs were put on the experimental diet for 10 days prior to the start of the experimental infections. The feed consisted of a mixture of star grass and lucerne hay and a concentrate made at the station. The concentrate was made by mixing 270kg of maize flour, 140kg of cotton seed cake and 70kg of maize germ and to this mixture molasses, vitamins and minerals were added. On average the concentrate contained 163g crude protein/kg dry matter (DM) and each lamb was allowed 0.25kg of the concentrate daily. Water was available *ad libitum.*

Remnants of the pre-weighed hay were collected daily and dried once a week at 105^0C for 72 hours (Sykes and Coop, 1976) so that the weekly group DM intakes could be calculated.

5.2.4 Methods

The parasitological, haematological, biochemical and statistical methods used are described in Chapter 2.

5.3 RESULTS

5.3.1 Mortalities

One lamb assigned to the infected Dorper group died of pneumonia two days after the start of the experiment. Five Dorpers and one Red Maasai died of haemonchosis from the evidence of weakness, generalised oedema, pallor of the mucus membranes and significant (>1500) post-mortem worm burdens (Table 5.1). Two other Dorpers had counts of only 330 and 610 worms and although they were weak prior to death and had some ascites and hydropericardium, the last PCV was above 20% and the cause of death was not clearly established. Three control lambs (one Red Maasai and two Dorpers) died of unknown causes and 20 adult *H. contortus* worms were recovered from one of these lambs.

5.3.2 Faecal egg counts

Control animals were mostly negative throughout the study but occasional egg counts of 50-100 epg were recorded in some animals.

Figure 5.1 shows the mean faecal egg output in the two infection groups. The egg output rose steadily from three weeks post infection to reach a maximum of 15,300 epg in the Red Maasai in Week 9. Thereafter it gradually fell to around 5000 epg from Week 15. In the Dorpers a first peak of 23,000 epg occurred in Week 9 and a second and higher peak of 32,000 epg in Week 12. Subsequently there was a rapid decline in egg output in the Dorpers and in the last few weeks the egg counts were similar in both breeds. The mean weekly counts (±S.D.) from patency through to the end of the experiment were 12,988 (11,971) for Dorpers and 8167 (10,538) for the Red Maasai and the difference was highly significant ($p<0.001$, Table 5.2). There were also highly significant individual animal and daily variations as well as significant breed by day interactions (Table 5.2).

5.3.3 PCVs

PCVs fell from means of 29% and 31% in Dorper and Red Maasai lambs

respectively in Week 1 to the lowest values of 17% in the Dorper in Week 12 and 22% in the Red Maasai in Week 10 (Fig. 5.2a). Thereafter the PCVs rose gradually and by the end of the experiment the levels were similar in both breeds. The difference due to breed was highly significant (Table 5.2). Individual animal variation, daily variations and breed by day interactions were also highly significant.

In control lambs of each breed the mean PCVs fluctuated between 25 and 30% (Fig.5.2b) and the difference was not significant.

5.3.4 Peripheral eosinophil counts

The mean eosinophil counts in peripheral blood, determined on seven occasions during the last eight weeks of the experiment are shown in Figure 5.3. The Red Maasai showed a greater response than the Dorpers and the difference was highly significant (Table 5.2). Much lower values were recorded in the control groups which had mean values (± S.D) of 0.11(0.19) and 0.15(0.16) in Dorper and Red Maasai lambs respectively; the difference was not significant.

5.3.5 Total serum protein

The mean total serum protein concentrations of the three samplings in Weeks 1, 9 and 13 were 52.2 and 58.8g/l in infected and control Dorpers and 56.3 and 60.3g/l in infected and control Red Maasai respectively. The difference between the two infected groups was significant ($p< 0.009$). The protein concentrations fell in the infected groups from a mean of 58.1g/l in Week 1 to 47.4g/l in Week 13 in the Dorpers and from 60.5 to 51.3 g/l in the Red Maasai but remained almost constant in the control groups (Fig. 5.4).

5.3.6 Weights and weight gains

The mean weights and weight gains are shown in Table 5.3. There were no significant differences in weight gains between the breeds or between groups. The Dorper control group had the highest mean weekly gain (0.33kg/animal) and

the Red Maasai infected, the least (0.17kg/animal). Gains for the Red Maasai control and Dorper infected groups were similar. Cumulative weight gains (Fig.5.5) obtained simply by adding successive mean weekly group gains reflect these observations. Comparison of the weight differences (last minus first weighing) between Dorpers and the Red Maasai showed a highly significant ($p<0.0003$, Table 5.4) difference with the Dorpers being heavier.

5.3.7 Dry matter intake

Infected groups consumed significantly more feed on a DM basis than their controls (Table 5.5).

Table 5.1: Total abomasal worm counts in lambs which died in the trickle infections experiment in which lambs repeatedly received 1000 *H. contortus* L_3

An. no.	Breed & treatment	Time of death*	Worm burdens			
			Adult *H. contortus*	Immatures	*T. axei*	Total
094	D,infected	2	-	-	-	-
062	D, "	36	280	30	20	330
092	D, "	46	390	220	0	610
096	D, "	56	4860	1080	0	5940
085	D, "	77	1650	120	0	1770
102	D, "	87	8560	580	0	9140
056	D, "	110	7340	210	0	7550
022	RM, "	110	6030	130	0	6160
048	D, "	129	4840	0	0	4840
033	RMcontrol	15	20	0	0	20
075	D, "	28	0	0	0	0
055	D, "	35	0	0	0	0

D= Dorper, RM= Red Maasai, * In days post the first infection

Table 5.2: Results of analysis of variance on log_{10}(epg+10), log_{10}(eosinophil count+1) and PCVs of Dorper and Red Maasai lambs repeatedly infected with 1000 *H. contortus* L_3

Source	Probability		
	EPG	PCV	Eosinophils
Breed	0.0013	0.0021	0.0006
Animal(breed)	0.0001	0.0001	0.0001
Day	0.0001	0.0001	0.0001
Breed*day	0.0001	0.0017	0.0208

Model: parameter= breed animal(breed) day breed*day. Parentheses indicate animal nested within breed while * indicates breed by day interaction

Table 5.3: Mean weights and weekly weight gains/animal in groups of Dorper and Red Maasai lambs repeatedly infected with 1000 *H. contortus* L_3 and in uninfected controls

Breed and Group	weight gains in Kg (S.D)	weight in Kg
Dorper infected	0.25 (1.2)[a]	19.2 (3.4)[1]
Dorper control	0.33 (1.0)[a]	19.7 (4.8)[1]
Red Maasai infected	0.17 (0.8)[a]	17.2 (2.4)[2]
Red Maasai control	0.26 (0.8)[a]	17.2 (3.5)[2]

Values with the same superscripts within a column are not significantly different. Those with different superscripts are significant ($p<0.001$)

Table 5.4: Results of analysis of variance on the difference of the last and the first weights of lambs of control and infected Dorper and Red Maasai sheep in the trickle *H. contortus* infections experiment

Source	Probability
Breed	0.0003
Infection	0.1285
Breed*infection	0.8660

Model: Weight difference= breed infection breed*time

Table 5.5: Mean weekly dry matter (DM) feed intake per animal of trickle *H. contortus* infected and control Dorper and Red Maasai lambs

Breed	Treatment	DM intake in g (S.D.)
Dorper	Infected	612.9^{a} (137.2)
Dorper	Control	512.9^{b} (91.7)
Red Maasai	Infected	458.8^{b} (72.1)
Red Maasai	Control	378.8^{c} (77.9)

Different superscripts indicate significant difference

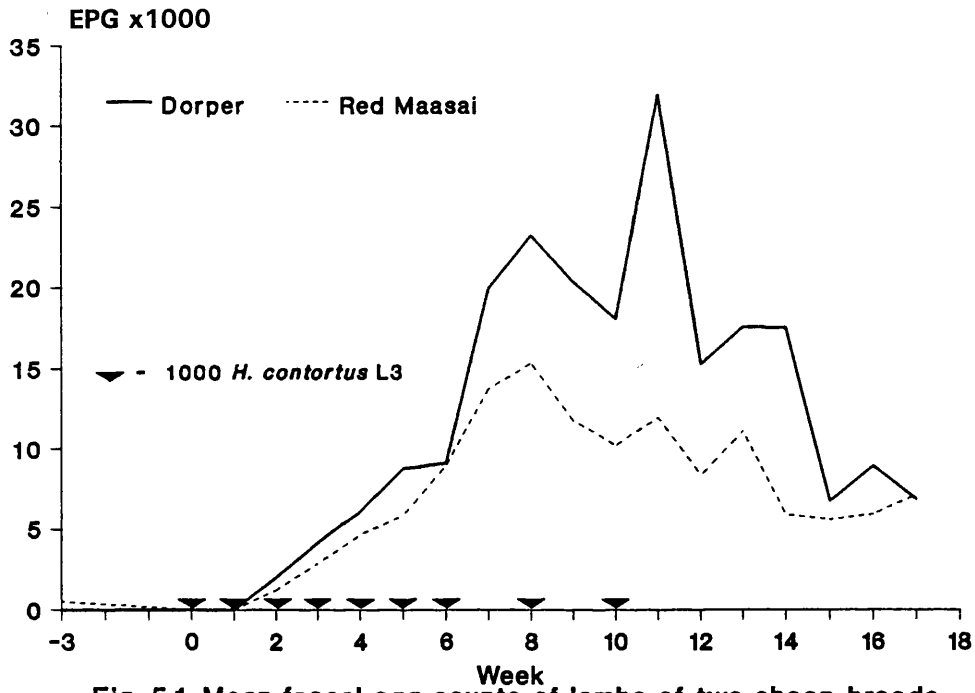

Fig. 5.1: Mean faecal egg counts of lambs of two sheep breeds repeatedly infected with *H. contortus* L3 from six months of age

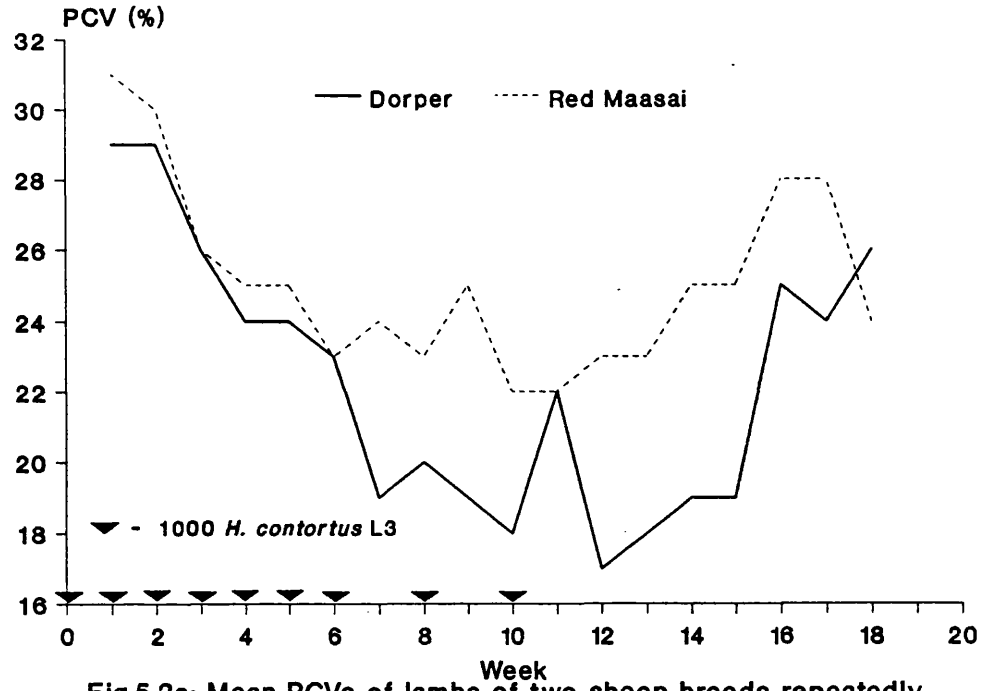

Fig.5.2a: Mean PCVs of lambs of two sheep breeds repeatedly infected with *H. contortus* L3 from six months of age

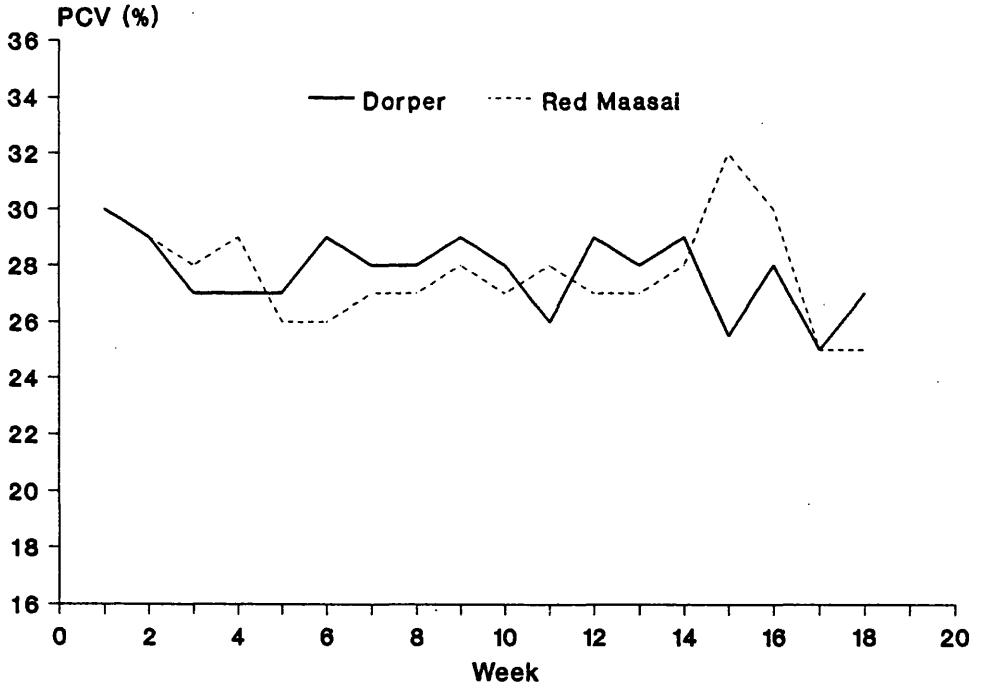

Fig.5.2b: Mean PCVs of uninfected lambs of two sheep breeds

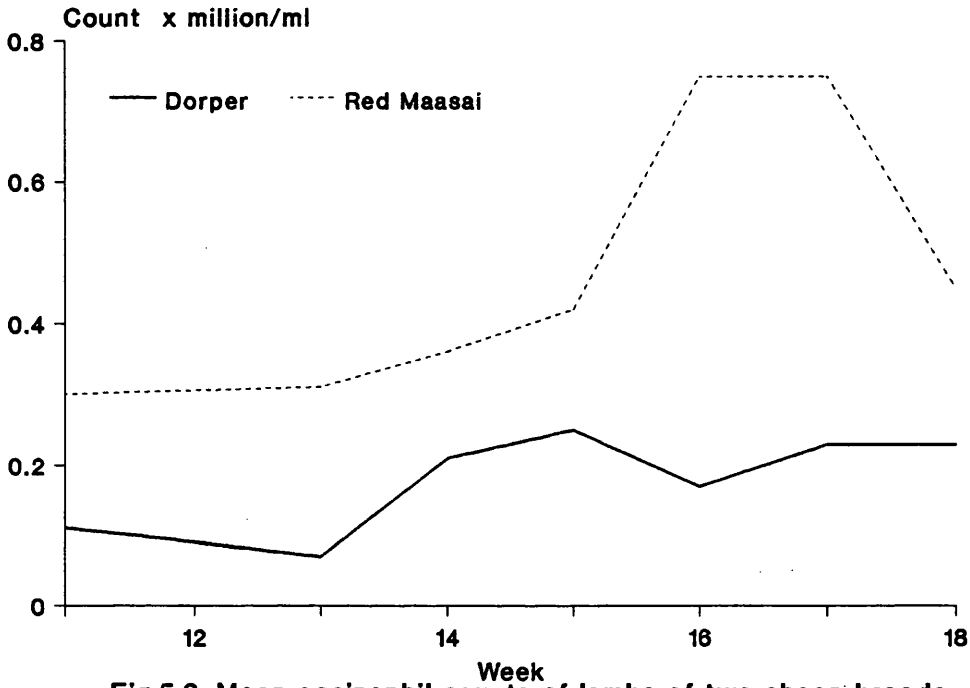

Fig.5.3: Mean eosinophil counts of lambs of two sheep breeds after a period of repeated infections with *H. contortus* L3

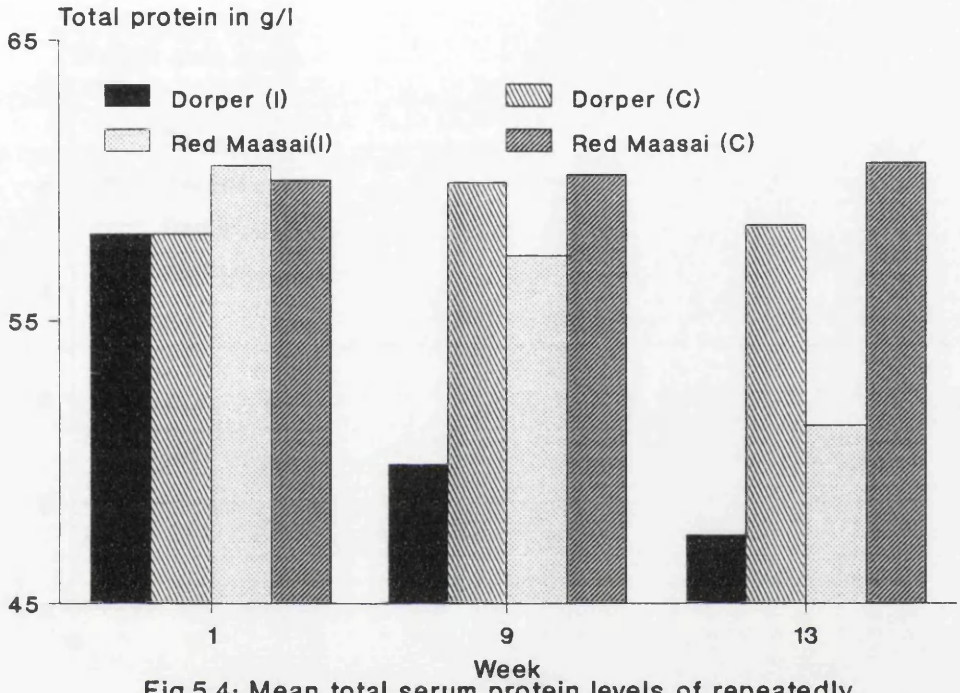

Fig.5.4: Mean total serum protein levels of repeatedly infected (I) and control (C) lambs of two sheep breeds

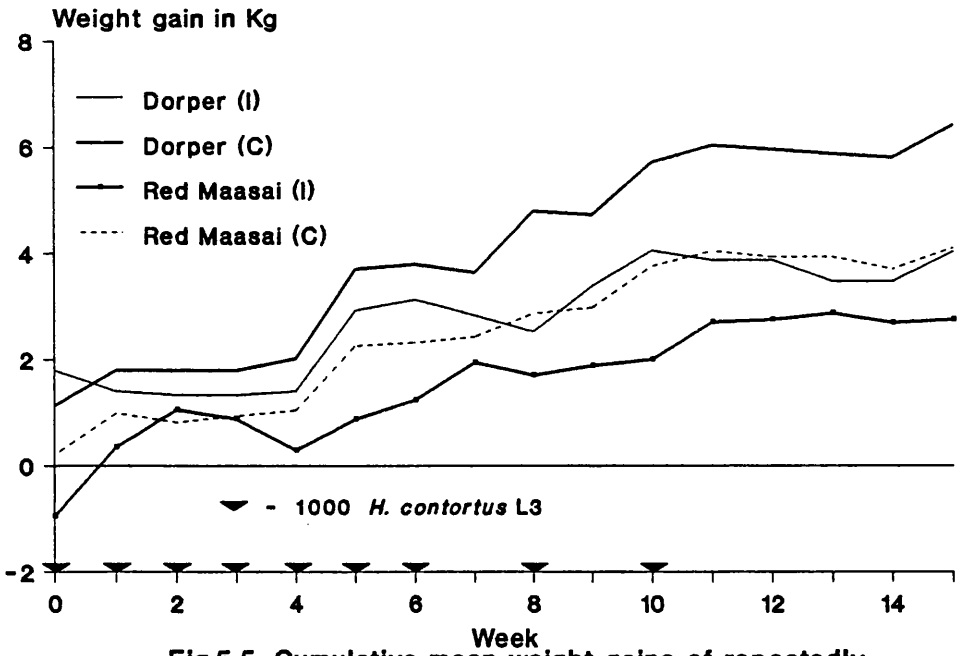

Fig.5.5: Cumulative mean weight gains of repeatedly infected (I) and control (C) lambs of two sheep breeds

5.4 DISCUSSION

The results showed clear differences between the Dorper and the Red Maasai in their susceptibility to a trickle infection with *H. contortus*. Faecal egg output was higher and more sustained in the Dorpers than in the Red Maasai except towards the end of the experiment when the counts were similar. This could be due partly to development of resistance in the Dorper with repeated infections, a common occurrence in trickle challenged sheep (Smith and Smith, 1993). It could also be partly due to the fact that five of the Dorpers which had heavy infections died. Since no necropsy worm counts were available at the end of the experiment, comparative resistance could only be assessed on the basis of mortality and faecal egg counts. Supportive evidence was derived from differences in PCVs, eosinophil counts and total serum proteins. Only one Red Maasai lamb died of haemonchosis compared to five Dorpers. The development of resistance after trickle infections has been observed by Abbott *et al.* (1988). These workers put four month old Dorset lambs on either a high or a low protein diet and then trickle infected them with *H. contortus* three times a week for 17 weeks after an initial single dose of 100 larvae/kg body weight. They found that lambs on the high protein diet developed resistance as judged by refractoriness to further infection and the return of PCVs to pre-infection values. In contrast lambs on the low protein diet did not develop any noticeable resistance.

The significantly higher egg output in the Dorpers in this study was accompanied by significantly lower PCVs and eosinophil counts. PCVs and eosinophil counts were not different between the two control groups indicating that the differences observed in the infected groups largely reflected differences in breed reaction to infection. In another experiment where Red Maasai and Dorper ewes were naturally infected in the field (Chapter 4), eosinophil counts were monitored once a week for 24 weeks. Significant differences in the counts occurred between the breeds only at certain times during the experiment. For example, significant differences occurred in the first two thirds of the experiment

when the Red Maasai mounted a higher response but towards the end of the study the Dorpers showed significantly higher responses. Throughout that field study, the Red Maasai ewes had significantly lower egg counts than the Dorper ewes.

Hypoproteinaemia is a common feature of helminth infections (Steel *et al.*, 1982; Abbott *et al.*, 1985a). Although only three observations on total serum protein were made, it appeared that infected Dorper lambs experienced a more severe hypoproteinaemia than the infected Red Maasai. The total serum protein concentrations fell from a mean of 58.1g/l in Week 1 to 47.4g/l in Week 13 in the infected Dorpers while at the same time concentrations fell from 60.5 to 51.3 g/l in infected Red Maasai lambs. No obvious changes in protein concentrations were recorded in uninfected control lambs of both breeds. Serum protein concentrations reported by Abbott *et al.* (1985a) showed a marked decrease in Finn Dorsets fed on a high protein diet and infected with a single dose of 125 *H. contortus* L3/kg bodyweight compared with minimal effects of the same infection dose on similarly fed Scottish Blackface lambs. However, a gradual decline in serum protein levels was observed in infected Scottish Blackface lambs on a low protein diet indicating that feed also has an effect. There were no noticeable changes in uninfected controls of either breed fed a high or low protein diet.

In the present study, there were no significant differences in weight gains between infected and uninfected control groups probably because of the high protein concentrate (163g/kg DM) and lucerne hay fed to the lambs. Similar findings have been reported by Smith and Smith (1993) who found no difference in weight gains between a trickle *H. contortus*-infected and an uninfected control group fed a high protein (145g/kg DM) diet. Abbott *et al.* (1988) also found no difference between control and infected groups of lambs on a high protein (170g/kg DM) diet. They also found that while anorexia was not present in the infected lambs on a high protein ration, anorexia was evident in the group fed the

low protein feed. In our experiment it appears that there was no anorexia in that infected groups consumed significantly more feed on a dry matter basis than their controls. However, reduction in the voluntary feed intake is a common feature of gastrointestinal nematodes although the reason for this is not known (Holmes, 1987). The degree of inappetence in lambs has been shown to vary with the level and duration of infection as well as with the level of protein in the diet (Abbott *et al.*, 1986, 1988).

Weight gains were lower, though not significantly so, in each infected group compared with its control. This could imply that uninfected animals were utilising feed better than their infected counterparts since the former were taking in less feed. However, this could be confirmed only if a second uninfected group was pair-fed to the infected group to allow the effect of parasitism on intake to be separated from that on food utilisation (Sykes and Coop, 1976, 1977). One lamb in the Dorper control group gained no weight over the duration of the experiment and remained at 12kg while another gained only 2kg. One lamb in the Red Maasai control group also gained only 2kg. Sykes and Coop (1976) attributed the inferior growth rate of sheep undergoing a trickle *T. colubriformis* infection more to reduced efficiency of food utilisation than to reduced appetite.

The feed intakes of the lambs in this experiment were very low compared with intakes of sheep used in other studies. For example, in the study of Sykes and Coop (1976) the DM feed intake of five month old Suffolk cross Greyface lambs was about 1526g/week/animal in the control group and 1442g/week/animal in *T. colubriformis* infected group which are about 2.5 times the intakes of the Dorpers in our study. However, the lambs were also twice as heavy. In another study the weekly feed intakes in *O. circumcincta* infected sheep was about 1113g/lamb and 1585g/lamb in the controls (Sykes and Coop, 1977) again 2 to 2.5 times that of the Dorper lambs in this study.

The overall mean weights of infected lambs and their controls were similar. Berry and Dargie (1976) found that in well-fed sheep, 200 *Fasciola hepatica*

flukes had no effect on either body weight or the capacity to gain weight in comparison to uninfected sheep. These workers observed that although live weight is easily measured, it is not the only index of productivity; carcass quality for example, which depends on the relative proportions of body solids and water may change independently of weight. Thus infected animals may apparently maintain weight but this could be due to the presence of oedema.

In conclusion, this experiment showed that Red Maasai lambs, obtained from a presumably diverse genetic pool, were more resistant than Dorper lambs after trickle *H. contortus* infections. The assessment was based on faecal egg counts, PCVs, eosinophil counts, total protein concentrations and mortality rates. Weight gains of infected animals were not depressed, most likely because infected animals were consuming more feed than their controls. The apparent absence of anorexia in the infected lambs was probably due to the palatability and high protein content of the ration fed to these animals.

CHAPTER SIX

RESPONSE OF DORPER AND RED MAASAI LAMBS TO PRIMARY, SECONDARY AND TERTIARY *HAEMONCHUS CONTORTUS* INFECTIONS

6.1 INTRODUCTION

Resistance to trichostrongyle infections in sheep varies depending on prior exposure, age and genetic constitution (Clunies Ross, 1932; Gregory *et al.*, 1940; Gamble and Zajac, 1992). Genetic variation of sheep in their susceptibility to nematode infections has been demonstrated in a number of studies where a sensitising infection is followed by challenge infection. Thus lower faecal egg counts after re-infection occurred in Merino sheep which had recovered from a single primary infection of 5000 *H. contortus* L3 (Adams and Beh, 1981). Between breed variation in response was also evident when four to six month old lambs were infected with 6000 *H. contortus* L3, treated after five to six weeks and re-infected with 12,000 larvae with pure bred St. Croix lambs showing a high degree of resistance to re-infection while cross-breds remained susceptible (Courtney *et al.*, 1985a).

Primary infections alone may result in variable responses which do not allow for discrimination between resistant and susceptible breeds (Courtney *et al.*, 1985a), lines (Gill, 1991) or individuals (Kassai *et al.*, 1990). For example, in the study by Kassai *et al.* (1990) classification of Merino sheep into 'low responders' and 'high responders' on the basis of worm counts was more reliable in animals given two infections than in those given only one infection. Adult worm counts carried out 50 days after the second infection appeared to reflect fairly accurately, the relative responsiveness of the hosts to infection.

In the experiment described in this chapter the responses of Dorper and Red Maasai lambs to three infections with 5000 *H. contortus* L3 administered at eight

to nine week intervals were investigated.

6.2 MATERIALS AND METHODS

6.2.1 Animals

Thirty three, four month old male Dorper lambs were purchased from Sosian ranch in Laikipia district at the beginning of August, 1992. Thirty two male Red Maasai lambs of about the same age were obtained from ADC Mutara ranch, also in Laikipia, and both groups brought to the N.V.R.C., Muguga. They were quarantined for two weeks and kept indoors for two months before they received the first *Haemonchus* infection.

6.2.2 Experimental design

The lambs were treated with ivermectin one month before the start of the experiment. Total abomasal worm counts were carried out in three Dorper and two Red Maasai lambs before anthelmintic treatment to provide an indication of the worm burdens originally present in the two groups.

During the experiment, the sheep pens were cleaned out twice a week to minimise the chances of extraneous infections.

Twenty lambs of each breed were infected with 5000 *H. contortus* L_3 in Weeks 0, 9 and 17. The experiment was terminated in Week 25. Five lambs of each breed were kept as extraneous infection controls while three worm-free Dorper and Red Maasai lambs served as larval infectivity controls when the main experimental groups received their second infections. A further two lambs of each breed were infectivity controls for the third infection.

Faecal egg counts were carried out weekly while PCVs and peripheral eosinophil counts were determined once a week until Week 9, twice a week for a further 12 weeks and then once a week for the remaining four weeks of the experiment. Serum samples were stored at -20^{0}C and assayed later for anti-*H. contortus* IgG, total protein and urea levels.

The lambs were sacrificed at the end of the study for total worm counts.

6.2.3 Feeding

The animals were fed on a mixture of star grass and lucerne hay. In addition, each lamb was allowed 0.25kg of a commercial pelleted concentrate per day for five days a week. The concentrate contained about 156g of crude protein/kg dry matter. Water was provided *ad libitum.*

6.2.4 Methods

The parasitological, haematological, biochemical, serological and statistical methods used are described in Chapter 2.

6.3 RESULTS

6.3.1 Mortalities

Three Red Maasai lambs died during the study. The first death, which occurred 14 days after the second infection was attributed to a non-parasitic enteritis and a total of 590 juvenile *H. contortus* were recovered. The second lamb died on Day 32 with a worm burden of 1260 adult *H. contortus* and the third lamb died 26 days after the second infection with a worm burden of 730 adults. These two lambs were weak with pale mucous membranes and at post-mortem, the abomasal mucosa was oedematous, there was some ascites and some hydropericardium. Their last PCVs were 18% and 19% respectively.

6.3.2 Faecal egg counts

Extraneous infection control lambs remained negative throughout the study.

After the first infection, the mean faecal egg count rose to a peak of 13,000 epg in the Dorpers in Week 5 followed by a higher peak of 16,400 epg in Week 8 (Fig.6.1a). In Week 9 when these lambs received the second infection, the egg

count had fallen to 10,200 epg and continued to decline to the lowest count of 1900 epg in Week 13. Thereafter, there was a rise to 11,200 epg in Week 17 at which time the third infection was given. After a dramatic fall to 2700 epg the following week, the egg counts fluctuated between 1660 epg and 5000 epg over the last seven weeks.

In the Red Maasai, the first peak of 5000 epg occurred in Week 5 and a second higher peak of 7300 epg in Week 7 (Fig.6.1a). Following the second infection in Week 9 a peak of 8200 epg occurred three weeks later. Subsequently the counts fell to 470 epg in Week 13, followed by a rise to 8500 epg in Week 17, at the time the lambs received the third infection. The counts declined to 1170 epg one week later only to rise to 7000 epg in Week 23.

Statistical analyses of the results from patency (Day 19) showed that there was no significant ($p<0.38$) breed difference but individual animal and daily variations were highly significant. There was also a highly significant breed by day interaction (Table 6.1).

6.3.3 PCVs

The mean PCVs fluctuated markedly in the two breeds (Fig.6.1b). After the first infection, the PCV in the Dorpers fell from 28% in Week 0 to 22% five weeks later (Fig.6.2a). Thereafter there was a general rise and the PCVs fluctuated between 23 and 29%. In the Red Maasai the PCV fell from 26% in Week 0 to 23% in Week 4 subsequent to which there was a general upwards trend and the PCVs fluctuated between 23 and 31% (Fig.6.2b).

Although the mean PCVs of the extraneous control lambs were generally higher than those of infected lambs, the changes followed a similar pattern (Fig. 6.2a, b).

Statistical analyses of PCVs of infected groups showed that there was no significant ($p<0.48$) breed difference. Individual animal variation and variation with time were, however, highly significant (Table 6.1). The breed by day

interaction was also significant ($p<0.02$).

6.3.4 Peripheral eosinophil counts

In the Dorpers there appeared to be a minor response one week after the first infection and a more obvious response one week after the second infection (Fig.6.3a). At the time of the third infection, eosinophil counts were increasing and they continued to do so to a high peak in Week 21 after which they showed a steady decline.

In the Red Maasai, an increase occurred five weeks after the first infection and a second increase, one week after the second infection (Fig.6.3b). As in the Dorpers eosinophil numbers were increasing at the time of the third infection and this increase continued to a peak in Week 21 before they subsequently declined. These obvious increases in eosinophil counts, observed after Week 16, also occurred in uninfected extraneous infection controls of the two breeds but differences between infected and control groups were more marked in the Dorpers (Fig. 6.3a, b).

Analysis of variance of eosinophil counts in the infected groups showed that there were no significant ($p<0.14$) breed differences. Individual animal variation and variation with time were, however, highly significant (Table 6.1). The breed by day interaction was not significant ($p<0.86$).

6.3.5 Haemoglobin types

All the lambs in this experiment were of the haemoglobin genotype AA.

6.3.6 Total serum protein

Total serum protein changes followed a similar pattern in infected animals of the two breeds (Fig. 6.4a). With the exception of Weeks 8 and 12 when the concentrations were the same in both breeds, the Red Maasai had the lower serum protein levels and towards the end of the experiment the gap appeared to

be widening. Serum protein concentrations in the control lambs of both breeds were very similar on the eight occasions tested (Fig.6.4a).

There was a significant ($p<0.02$) breed difference and highly significant individual animal and daily variations (Table 6.2). The breed by day interactions were also highly significant ($p<0.009$).

6.3.7 Urea

Urea concentrations were remarkably similar in infected and control animals of both breeds (Fig. 6.4b). Urea concentrations appeared to increase following both the primary and secondary infections while the third infection appeared to have little or no effect on the concentrations. Overall, the urea concentrations increased from a pre-infection level of 4.1 to 8.1 mmol/l in the Dorpers at the end of the experiment and from 4.5 to 8.0 mmol/l in the Red Maasai. Urea concentrations in the control lambs increased in a similar fashion and to the same levels observed in the infected lambs (Fig. 6.4b).

Results of statistical analyses (Table 6.2) showed no significant ($p<0.87$) breed difference and while individual animal and daily variations were highly significant, the breed by day interaction was not.

6.3.8 ELISA O.D.s

There appeared to be some increases in ELISA O.D. values after each infection using either adult worm or larval antigen (Fig. 6.5). The mean O.D. values were higher with larval antigen and values in the Dorpers were generally higher than in the Red Maasai.

Only individual animal variations and variation with day were significant with either antigen (Table 6.3).

6.3.9 Total abomasal worm counts and worm lengths

The abomasal worm counts of the five sheep which were necropsied prior to the

experimental infections are shown in Table 6.3. The worm load was low and consisted of both *H. contortus* and *T. axei*.

There was no significant difference in total abomasal worm counts at necropsy between the two breeds (Table 6.4). The counts ranged from 60 to 1690 in the Dorpers and from 0 to 2170 in the Red Maasai.

No significant differences in the lengths of worms obtained from the two breeds were found but significant individual variations occurred (Table 6.4).

Table 6.1: Results of analysis of variance of faecal egg counts, PCVs and eosinophil counts of Dorper and Red Maasai lambs infected three times with 5000 *H. contortus* L3 at eight to nine week intervals

Source	Probability		
	Log_{10}EPG	PCV	Log_{10}Eosinophils
Breed	0.3854	0.4800	0.1367
Animal(breed)	0.0001	0.0001	0.0001
Day	0.0001	0.0001	0.0001
Breed*day	0.0001	0.0174	0.8646

Model: Parameter= breed animal(breed) day breed*day

Parentheses indicate animal nested within breed and * indicates breed by day interaction

Table 6.2: Results of analysis of variance of total serum protein, urea and ELISA O.D. values with adult worm or L3 antigens in Dorper and Red Maasai lambs infected three times with 5000 *H. contortus* L3 at eight to nine week intervals

Source	Total protein	Urea	Antigen	
			adult	L3
Breed	0.0227	0.8719	0.9525	0.3450
Animal(breed)	0.0001	0.0001	0.0001	0.0001
Day	0.0001	0.0001	0.0137	0.0019
Breed*day	0.0088	0.5145	0.7177	0.9257

Model as in Table 6.1

Table 6.3: Total abomasal worm counts in lambs which died or were sacrificed prior to the start of experimental infections with *H. contortus*

Animal no.	Breed	Species		Total
		H. contortus	*T. axei*	
172	Dorper	30	20	50
112	Dorper	60	0	60
119	Dorper	60	20	80
271	Red Maasai	20	140	160
285	Red Maasai	50	10	60

Table 6.4: Results of analysis of variance of total worm counts and worm lengths at necropsy, of Dorper and Red Maasai lambs infected three times with 5000 *H. contortus* L3 at eight to nine week intervals. The mean counts and lengths and their S.D.s (in parentheses) are also shown

Breed	Mean worm count	Mean length (mm)	
		Females	Males
Dorpers	585(412)	23.7(2.0)	15.9(1.3)
n	*19*	*85*	*78*
R/Maasai	671(678)	24.4(2.6)	15.8(1.4)
n	*17*	*99*	*93*

	Probability		
	Worm burdens	Worm lengths	
		females	males
Breed	0.537	0.1890	0.9216
sheep(breed)		0.0002	0.0188

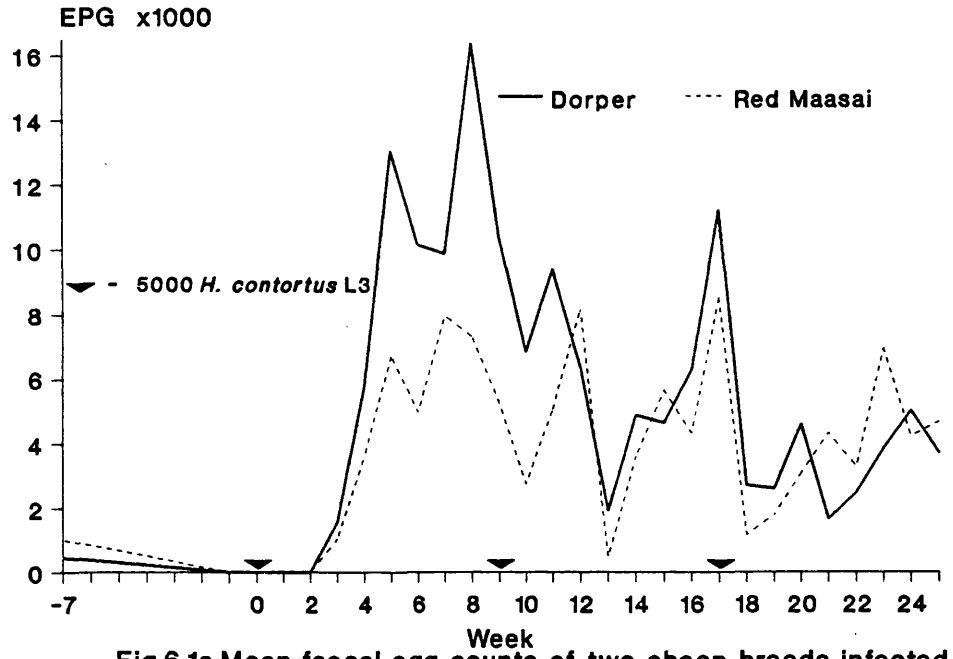

Fig.6.1a:Mean faecal egg counts of two sheep breeds infected with three doses of *H. contortus* L3 from six months of age

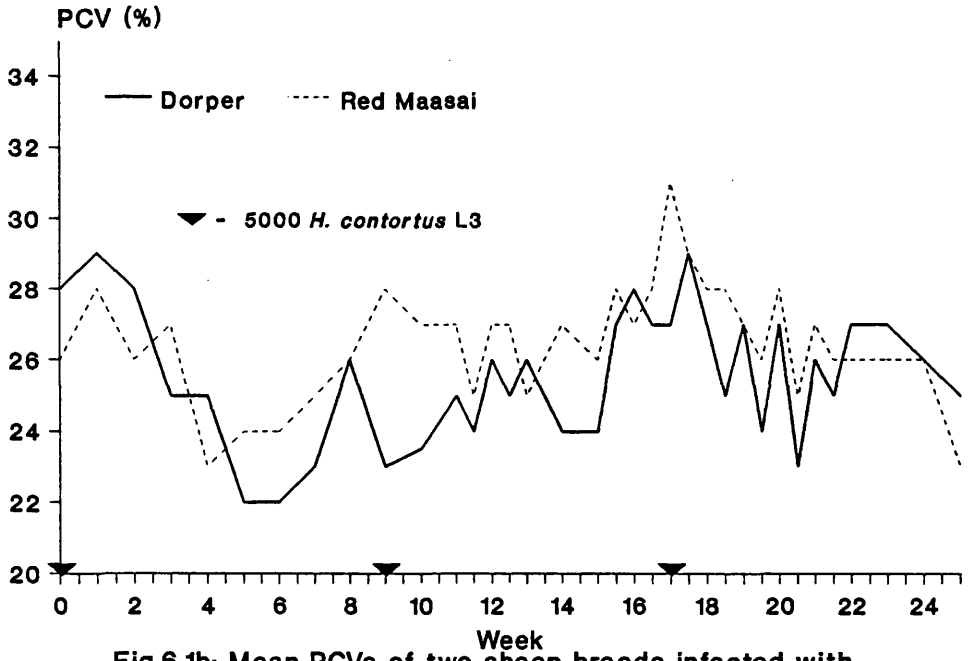

Fig.6.1b: Mean PCVs of two sheep breeds infected with three doses of *H. contortus* L3 from six months of age

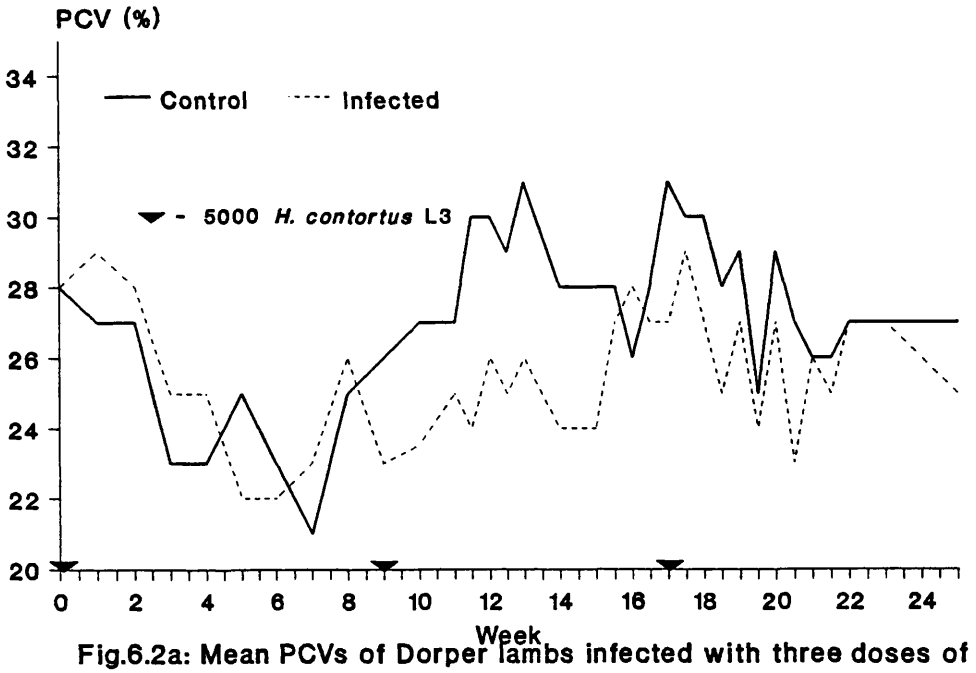

Fig.6.2a: Mean PCVs of Dorper lambs infected with three doses of *H. contortus* L3 from six months of age and uninfected controls

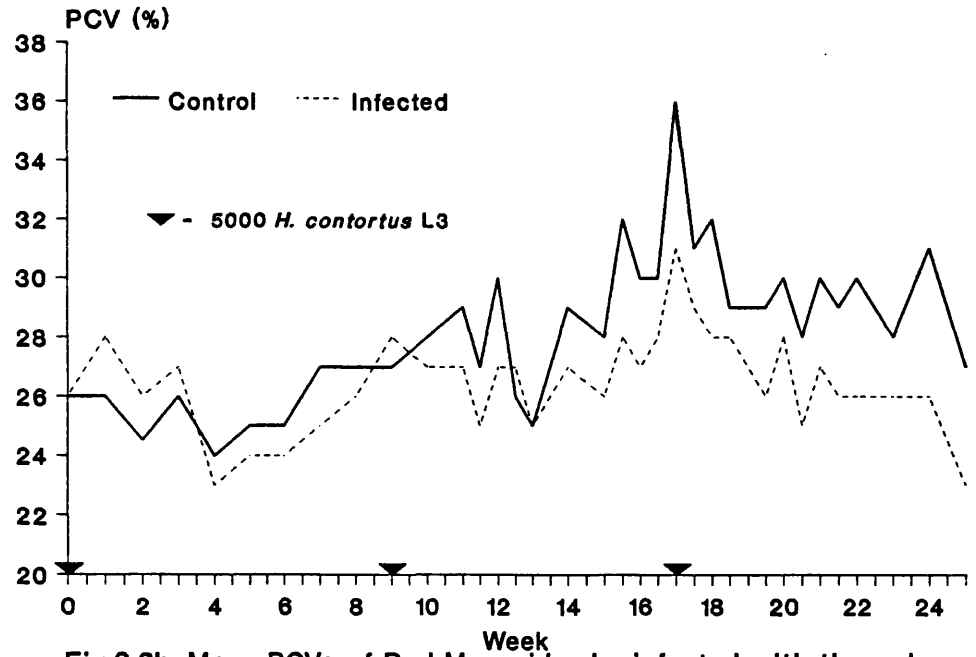

Fig.6.2b: Mean PCVs of Red Maasai lambs infected with three doses of *H. contortus* L3 from six months of age and uninfected controls

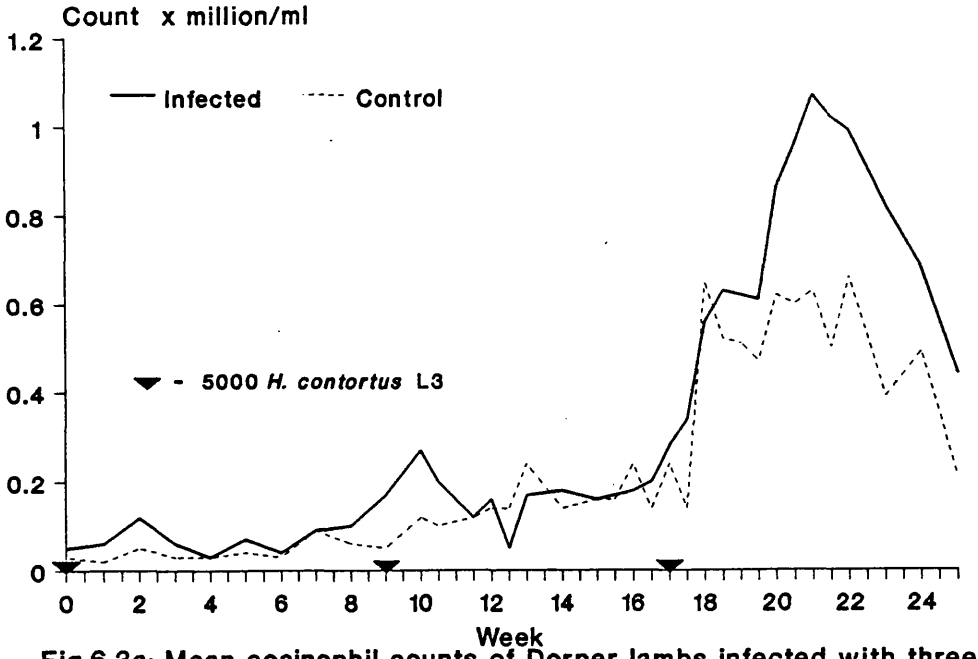

Fig.6.3a: Mean eosinophil counts of Dorper lambs infected with three doses of *H. contortus* L3 from six months of age and uninfected controls

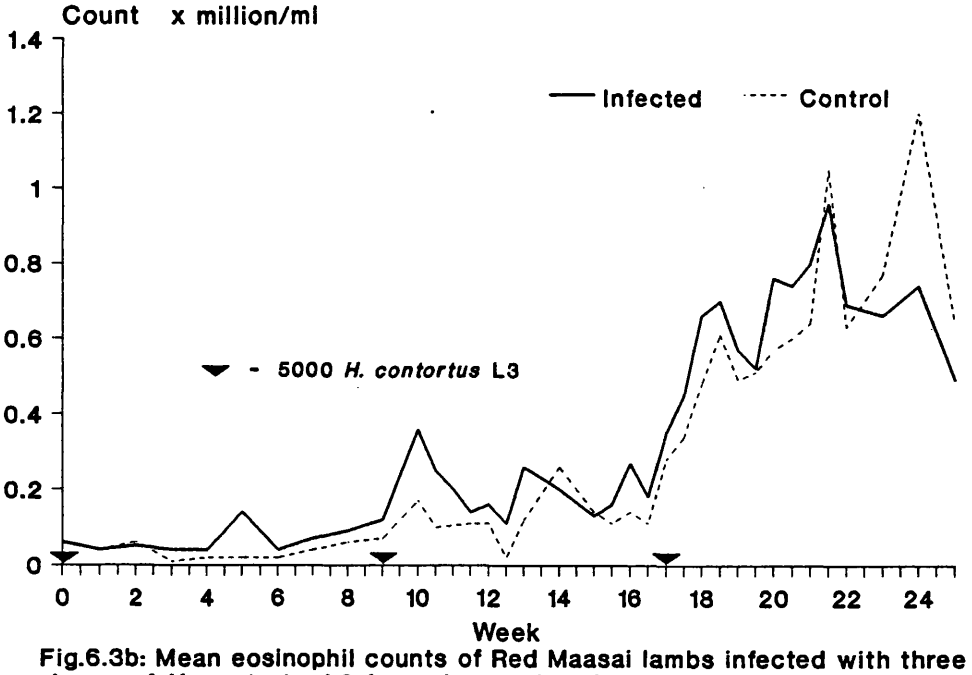

Fig.6.3b: Mean eosinophil counts of Red Maasai lambs infected with three doses of *H. contortus* L3 from six months of age and uninfected controls

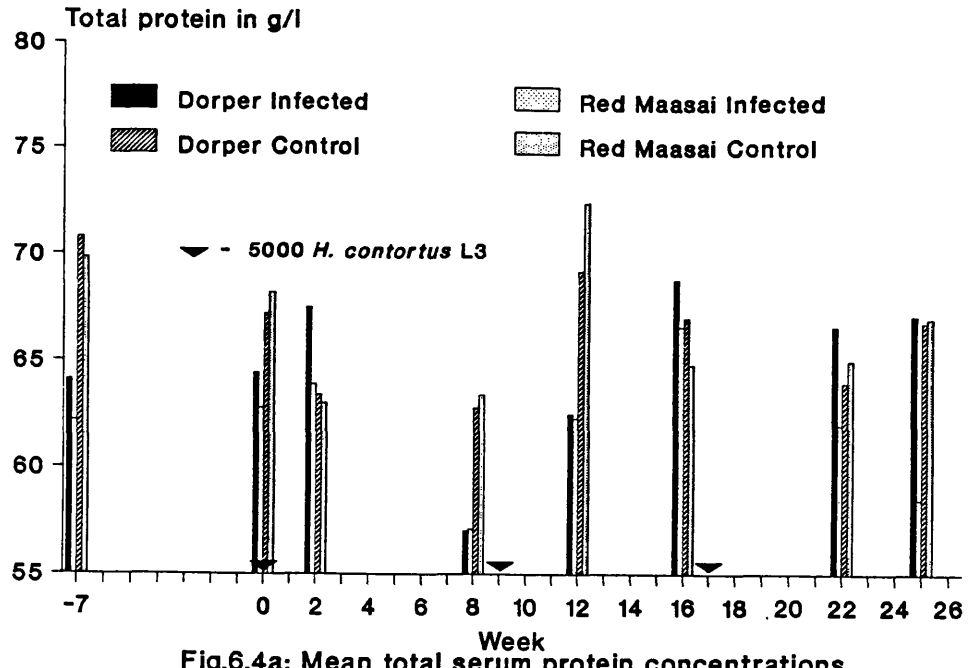

Fig.6.4a: Mean total serum protein concentrations of infected and control lambs of two sheep breeds

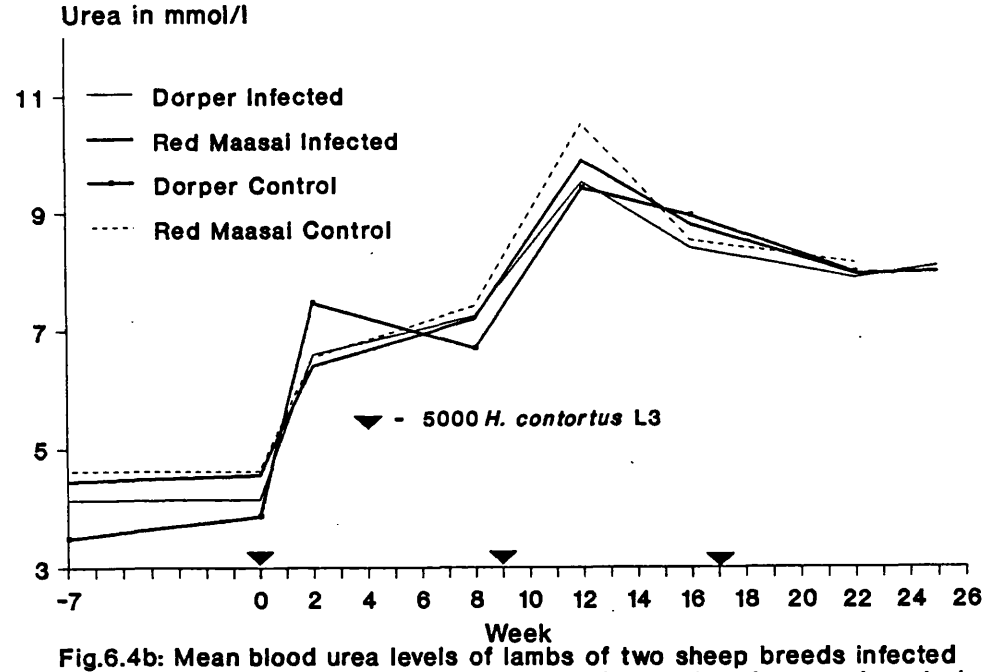

Fig.6.4b: Mean blood urea levels of lambs of two sheep breeds infected with three doses of *H. contortus* L3 from six months of age and controls

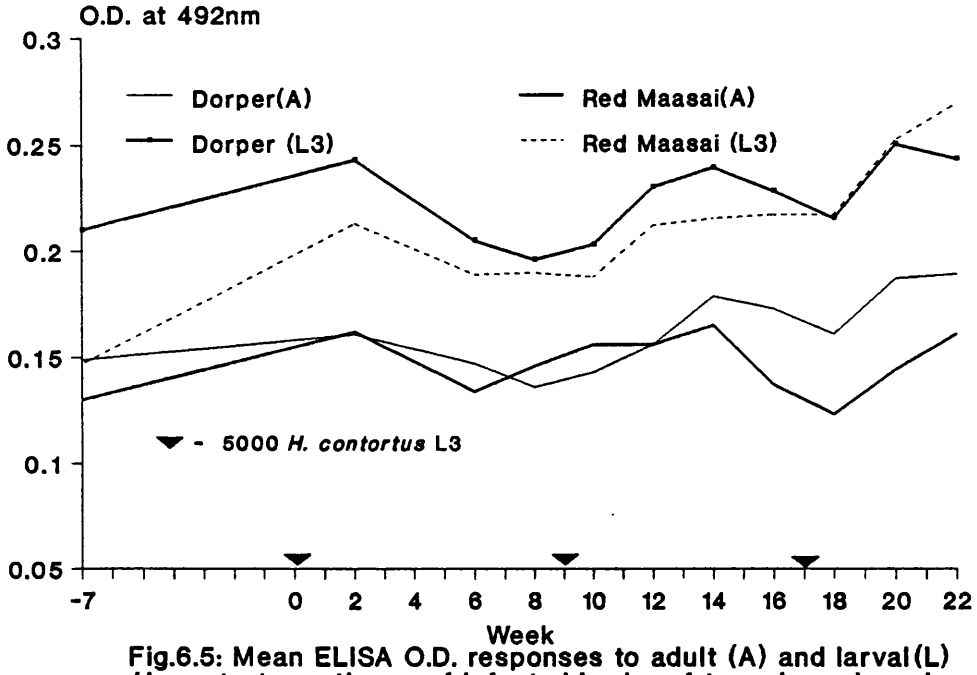

Fig.6.5: Mean ELISA O.D. responses to adult (A) and larval(L) *H. contortus* antigens of infected lambs of two sheep breeds

6.4 DISCUSSION

In this experiment there was no difference in resistance between the two breeds on the basis of faecal egg counts, PCVs and total worm burdens at necropsy in contrast to the results of the first three experiments where the Red Maasai was more resistant to *H. contortus* infection than the Dorper. However, the fact that there was a highly significant breed by time interaction indicated that at certain times there were significant differences in faecal egg counts. The most obvious such time was from patency to Week 11 when the Dorpers had higher egg counts than the Red Maasai. These results were also at variance with earlier published reports (Allonby, 1975; Castellino, 1976; Preston and Allonby 1978, 1979a) in which the Red Maasai was more resistant to haemonchosis than several other breeds after either natural or artificial infection. Judging from the worm burdens of lambs necropsied prior to the start of the experiment and the faecal egg counts pre-treatment, the level of prior exposure in both breed groups was relatively low. It may be that the Red Maasai only shows superior resistance relative to other breeds after either a higher or a sustained early exposure. However, there is evidence in Merino sheep which suggests that early heavy infections are likely to induce immunological tolerance leading to increased susceptibility to re-infection, especially in *Haemonchus* endemic areas like Kenya (Lopez and Urquhart, 1967; Allonby, 1975). Perhaps a more plausible explanation is that when the Dorper is well fed as was the case in this experiment, it will be as resistant as, or even more resistant than the Red Maasai. This might have been the case judging from the studies of Fourie (1931) and of Wilson and Samson (1974) who encountered difficulties reproducing clinical haemonchosis in well-fed sheep. It is noteworthy that whereas no Dorper died, two Red Maasai died of what appears to have been chronic haemonchosis. That the Dorper showed similar resistance to the Red Maasai with good feeding is in contrast to the findings of Preston and Allonby (1978) who reported that the relative susceptibilities of Hampshire Down, Corriedale, Merino and Red Maasai sheep

were maintained whether the plane of nutrition was high or low. This is also in contrast to the findings reported earlier (Chapter 5) where, on a high protein diet, the Red Maasai was still superior to the Dorper following trickle *H. contortus* infections. It is difficult to compare diets between studies but it is possible that the sheep in the present experiment were receiving more dietary protein than those in the study by Preston and Allonby (1978) since they were fed concentrates in addition to lucerne hay. Regarding the differences between this and the earlier trickle infection study, it may be that the trickle infections were more pathogenic than a single infection and that the Red Maasai is better able to resist the ravages of infection than the Dorper. Although in some studies single infections have been found to be more pathogenic than trickle infections (Andrews, 1942; Manton *et al.*, 1962; Dineen *et al.*, 1965), pathogenicity can be similar (Abbott *et al.*, 1988). In this context Pradhan and Johnstone (1972a, b) found daily infections to be more pathogenic than weekly infections although the total dosage was the same. However, in the present study there is no evidence to support this speculation and in fact the results of the following chapter tend to indicate the contrary. A better response to small continuous infections appears to be a more reasonable explanation and it may be partly the reason for the superior resistance shown by the Red Maasai in the two field studies where infections were naturally acquired presumably in a trickle fashion. In addition, there are cases where a breed has shown superior resistance at one time and failed to do so at another. For example, when the numbers of nematode parasites naturally acquired by lambs of five breeds born in 1969 were compared, there were no significant breed differences. However, when lambs of the same five breeds born the following year were compared, Navajo lambs had significantly fewer *H. contortus* burdens than lambs of the other four breeds (Knight *et al.*, 1973).

In this experiment both breeds responded to the first infection with the Dorpers showing higher faecal egg counts. The second infection appears to have established successfully in the Red Maasai since there was a rise in faecal egg

counts three weeks later. In the Dorpers, however, although there was an apparent rise in faecal egg counts two weeks after infection the general trend was downwards. The steady increase in faecal egg counts noted in both breeds after Week 13 cannot be attributed to the normal development of the second infection. It suggests either a very slow maturation of worms acquired from that infection or a delay in ovulation. It could also indicate a temporary increase in the fecundity of adult worms (Dineen *et al.*, 1965). The highly significant ($p<0.0001$) breed by infection variation (not presented) showed that the response to each infection was different and supports the observations made above.

In general, faecal egg counts appeared to diminish with time despite the superimposed second and third infections. Failure of faecal egg counts to increase following a second and third infection can occur in experimentally infected sheep but there may be individual animals which will remain susceptible (Adams and Beh, 1981). This refractoriness to re-infection is associated with development of immunity due to prior exposure and with increasing age of the host.

A self-cure appears to have occurred in the two breeds following the third infection since the egg counts fell by 76% and 86% within a week in Dorpers and the Red Maasai respectively.

As with all other parameters measured, there was a significant individual variation in worm lengths. The level of significance was much higher with female than with male worms implying that the host's effects on the parasites can differentially affect one sex more than the other. Similarly, in sheep immunised with an integral gut membrane protein from adult *H. contortus*, there was a greater reduction in the number of females than of males at necropsy (Munn *et al.*, 1993; Smith *et al.*, 1993).

The PCVs in the infected and control Dorper and Red Maasai groups fell after the experiment started probably due to a combination of infection and regular sampling since a fall has also been reported in regularly bled uninfected control

lambs (Abbott *et al.*, 1985a). Consistent with lack of a significant difference in faecal egg counts between the breeds, there was no significant difference with PCVs. However, the breed by time interaction was again highly significant indicating that there were significant differences at certain periods in the course of the study. Despite the generally good body condition of the sheep in this experiment, the PCVs were rather low. Conway and Whitlock (1965) observed that sheep can adjust to such levels of PCVs if not stressed by cold or exercise and that sheep at rest may selectively sequester erythrocytes to effect these levels.

No breed differences in eosinophil counts occurred, consistent with lack of differences in faecal egg counts and total worm counts. There was also no significant breed by time interaction implying that the breeds behaved similarly in their eosinophil responses. In this experiment the secondary response was a little higher than the primary response in both breeds. Eosinophilia is T-cell dependent (Sewell and Vadas, 1983) and therefore an anamnestic response is likely following secondary exposure to antigen, as in acquired humoral or cellular immunological responses. Thus intravenous inoculation of *T. spiralis* larvae into rats resulted in eosinophilia with a peak on Day 6 and the response was enhanced on re-injection of the parasites 20 days later (Boyer *et al.*, 1970). Irradiated rats infected with *T. spiralis* larvae failed to develop eosinophilia unless they were infused with both normal bone marrow suspension and lymphocytes (Basten and Beeson, 1970). Furthermore, when the lymphocytes used were obtained from donors infected five weeks previously, a secondary type-reaction developed showing that the lymphocytes had 'memory' for eosinophilia. The response can also be abolished in rats using immunosuppressive agents (Boyer *et al.*, 1970). The more marked eosinophilia which occurred following the third infection in the present study was not entirely due to infection since counts were rising at the time of this infection and uninfected control animals of both breeds showed similar increases to the

infected groups. This suggests that there was some other eosinophilia-stimulating factor operating in all of the animals. This may have had a physiological basis since eosinophilia can occur in response to histamine released following the degradation of mast cells (Schalm *et al.*, 1975) and mast cells may increase substantially, for example in the bovine during oestrous (Weber *et al.*, 1950).

Although the total serum proteins were not analysed statistically to compare levels in control and infected animals, the control animals had higher levels between Weeks 5 and 15 post infection. They also showed higher levels in the two pre-infection samples. The significantly lower protein concentration in the Red Maasai, especially in the course of the first infection, does not appear to be entirely related to infection since the Red Maasai had lower faecal egg counts during this period and there were no differences in PCVs between the two breeds. The lowest total protein concentrations in both breeds appeared to coincide with the first peaks of faecal egg counts following the first infection.

Urea, which is the main end-product of protein metabolism, is elevated in renal disease and some other disorders but high protein diets may also cause elevated blood urea levels in normal animals (Benjamin, 1978; Doxey, 1983). Thus Abbott *et al.* (1986) found mean urea concentrations of 9 mmol/l in uninfected sheep on a high protein diet while in the corresponding low protein group the value was only 5 mmol/l. In the same study, an increase in blood urea concentration was seen in two *H. contortus* infected groups fed either a high or a low protein diet. This increase occurred from the third week post- infection. Differences between infected and control lambs on the high protein diet were only significant in Week 5 while differences between infected and control lambs on the low protein diet were significant between Weeks 3 and 6. This study therefore suggested that blood urea levels were affected by both diet and infection. Elevated levels may also occur, for example, as a result of increased protein catabolism secondary to starvation, small intestinal haemorrhage, necrosis and prolonged exercise (Duncan and Prasse, 1986). Increases in urea

levels in helminth infections have also been documented by Parkins *et al.* (1973) and normal seasonal variations are known to occur (Sykes and Russel, 1979). The normal ovine concentrations vary from 2.6 to 7.6 mmol/l with a mean of 4.6 mmol/l (Doxey, 1983) although the range may be wider. In the present experiment, the reason for the increased urea concentrations seen with the onset of regular sampling in both infected and uninfected animals is not known. However, the minimal differences between control and infected animals agree with the findings of Abbott *et al.* (1986) that with an adequate protein diet the differences between control and infected sheep are not obvious.

Serum antibody levels are often poorly correlated with resistance to *H. contortus* infections in terms of either worm burdens or faecal egg counts (Smith, 1977b; Adams and Beh, 1981). In the present experiment where there were no differences in resistance between Dorpers and Red Maasai lambs, there were no differences in serum antibody responses between the breeds. In the experiment described in Chapter 3 the resistance shown by the Red Maasai in terms of faecal egg counts was accompanied by significantly higher serum antibody levels. Also in *T. colubriformis* infections, Windon and Dineen (1981) reported that complement fixing antibodies were the most reliable indices of resistance in Merino sheep selected for increased or decreased resistance. These facts suggest that IgG has a role to play in resistance but this remains to be elucidated. The higher responses obtained with L_3 than with adult worm antigen was also reported by Cuquerella *et al.* (1991) with Manchego lambs.

In conclusion, in this experiment where six month old Red Maasai and Dorper lambs were infected three times with 5000 *H. contortus* L_3 at eight to nine week intervals, there was no apparent difference in resistance between the two breeds in terms of either faecal egg counts or total worm burdens. However, there were times, especially following the first infection, when the Red Maasai lambs shed significantly fewer eggs. This lack of any significant difference was reflected in the PCV, eosinophil and serum IgG results. Although there was a marked

eosinophilia during and after the third infection this was probably partly due to some physiological phenomenon since it occurred at the same time in the uninfected control lambs. Urea levels were found to be similar in both control and infected lambs probably due to the high plane of nutrition.

CHAPTER SEVEN

EXPERIMENTAL *HAEMONCHUS CONTORTUS* CHALLENGE IN TWO AGE GROUPS OF DORPER AND RED MAASAI LAMBS

7.1 INTRODUCTION

Exposure to infection with *Haemonchus contortus* conveys a substantial degree of immunity to re-infection in adult sheep but lambs under seven months of age show varying degrees of immuno-competence and generally show little resistance to repeated infections. For example, a considerable degree of immunity to a challenge infection of 15,000 L_3 developed in 10-12 month old Down lambs given a total of 9000 normal *H. contortus* larvae each, either in two equal doses four weeks apart or in 30 doses of 300 larvae at two day intervals (Manton *et al.*, 1962). In the same experiment, and with the same regimens, two to four month old lambs were not protected from a challenge infection of 5000 larvae after exposure to a total primary dose of 3000 larvae. In contrast Christie and Brambell (1966) induced a relatively high level of resistance to *H. contortus* challenge in two to three month old Scottish Blackface cross lambs by first exposing them to serial doses of infective larvae followed by anthelmintic treatment. Six daily doses of 25,000 larvae were given followed by treatment with thiabendazole; commencing a week later a further eight daily infections were administered which were also terminated by anthelmintic treatment. The mean worm burden after challenge with 50,000 larvae divided and given in two doses of 25,000 at an interval of one hour was 3.5 times higher in the challenge controls than in the previously sensitised group. However, in an earlier experiment with similarly bred eight month old Scottish Blackface cross lambs given 20,000 larvae daily for 10 days followed by anthelmintic treatment, the level of protection to challenge with 51,000 larvae in three divided doses given over a 24

hour period was lower (Christie et al., 1964). This discrepancy was largely attributed to the more variable susceptibility shown by the challenge control group in the experiment with the older lambs.

Genetic variation of sheep in susceptibility to nematode infections is well recognised (Piper, 1987). For example when four to six month old lambs were infected with 6000 *H. contortus* larvae, treated with levamisole after five to six weeks and re-infected with 12,000 larvae, St. Croix lambs were found to be highly resistant to re-infection while cross-bred lambs remained susceptible (Courtney et al., 1985a). Even when infected as early as two months of age, St Croix lambs developed significantly greater levels of resistance to *H. contortus* than Dorsets of the same age (Gamble and Zajac, 1992). In the latter studies where the lambs were infected with 500 larvae five days a week for six weeks then treated with fenbendazole before challenge, age and previous exposure were found to influence the level of resistance.

Almost twenty years ago studies on the Red Maasai sheep showed that this breed was more resistant to haemonchosis than several other breeds exotic to Kenya. Allonby (1975) reported that the Red Maasai was more resistant to experimental *H. contortus* infection than the Dorper, Corriedale and Merino based on faecal nematode egg counts, packed cell volumes (PCVs) and weight gain; the ages of the sheep in these studies were not given. Subsequent studies by Preston and Allonby (1978, 1979a) with adult sheep showed that the Red Maasai was also more resistant to haemonchosis than the Hampshire Down and Blackhead Persian. Also in Kenya, Castellino (1976) serially necropsied Red Maasai and Merino sheep on Days 7, 12, 15, 18, 21, 23, 26 and 28 after an experimental infection with a single dose of 250 *H. contortus* L_3 /kg body weight. Two animals of each breed were killed on each occasion. Although it was found that the worm establishment in the two breeds was comparable, after Day 15 of infection, the worm recoveries in the Red Maasai fell

below 10 percent of the larval dose administered. Burdens in the Merino also showed a fall but were well over 20 percent. A further experiment with parasite-naive lambs was carried out to determine whether the early loss of infection in the Red Maasai had been influenced by prior exposure because the animals used in the first experiment were not reared worm-free from birth. The results were similar to those of the first experiment but loss of infection was delayed by 10 days, that is until Day 25. It was concluded that the Red Maasai were able to get rid of an infection more effectively than the Merino and that previous experience enhanced the response. In both studies, however, the ages of the experimental sheep were not indicated.

The purpose of the study described here was to investigate whether there were age-related differences in susceptibility to repeated *H. contortus* infections in Dorper and Red Maasai lambs. Repeated infections were chosen since the results of a primary infection in terms of faecal nematode egg counts, PCVs and total worm counts at necropsy are very variable compared with those following secondary infection (Courtney et al., 1985a; Kassai et. al., 1990). Groups of Red Maasai and Dorper lambs were given a primary infection at either four or six months of age. All animals received two challenge infections at intervals of eight to nine weeks.

7.2 MATERIALS AND METHODS

7.2.1 Animals

Red Maasai and Dorper lambs born at the NVRC, Muguga in October 1992 were randomly divided into two groups when they were four months old. Group 1 consisted of 21 Dorpers (12 females, 9 males) and 23 Red Maasai (12 females, 11 males); these were used for the first experimental infection. Group 2 had 21 Dorpers (12 females, 9 males) and 29 Red Maasai (13 females, 16 males) which were infected when six months old. The males were not castrated. Although the lambs

were reared under conditions designed to prevent nematode infection, 18 Dorper lambs of Group 1 had stronglye egg counts ranging from 50-18,000 epg while 12 Red Maasai had counts ranging from 50-200 epg 25 days before the experimental infection. At the same time in Group 2, eight Dorper lambs were infected with counts varying from 100-8300 while five Red Maasai lambs had counts ranging from 50-2900 epg. The source of infection was not established. The lambs had negative faecal egg counts at the first screening 25 days earlier. Eighteen days before the experimental infection all the lambs were treated with a cattle preparation of ivermectin (Ivomec^{R}, MSD Haarlem, Netherlands) administered subcutaneously and each lamb received 5mg.

7.2.2 Experimental design

Each animal in Group 1 was given 5000 *H. contortus* L_3 larvae orally at four months of age and monitored weekly for faecal nematode egg output, PCVs and eosinophils. Group 2 acted as uninfected controls and were similarly monitored at fortnightly intervals to check for extraneous infection and for any haematological changes which might occur independent of infection. The Group 1 animals received a second infection after eight weeks at which time the Group 2 animals were given their first infection thus serving as larval infectivity controls. All of the animals were monitored weekly for another nine weeks when Group 1 and Group 2 were re-infected with 5000 L_3 for the third and second time respectively. At this point four additional worm-free lambs were similarly infected to serve as larval infectivity controls and another four worm-free lambs were kept as uninfected controls. Monitoring continued weekly as before. Nine weeks later the Group 2 animals were infected for a third time with 5000 L_3 larvae each. At this stage three worm-free lambs were again similarly infected to serve as larval infectivity controls. At this time Group 1 animals were necropsied for total worm counts. The Group 2 lambs

were necropsied 13 weeks after their third infection.

Haemoglobin typing was carried out after the lambs had already been allocated to the groups.

7.2.3 Feeding

Animals were fed on hay and received approximately a quarter of a kilogram of commercial young stock pelleted concentrates per head per day five days a week. Water was available *ad libitum.*

7.2.4 Methods

The parasitological, haematological serological and statistical methods used are as described in Chapter 2.

7.3 RESULTS

7.3.1 Group 1: four month old lambs

Haemoglobin types

Only two lambs, both Red Maasai males were of haemoglobin type AB. All the other lambs were of haemoglobin type AA.

Mortalities

Two lambs died during the first infection period, a Dorper male which died of an unknown cause two days after the first infection and a Red Maasai female which died 27 days after infection. At post-mortem 2220 adult *H. contortus* worms were recovered from this lamb.

During the second infection period an additional four Red Maasai lambs died (one female and three males). Their worm counts (last PCVs) were 820(23), 1140(20), 2050(14) and 2530(11). After the third infection a further six lambs died (one

Dorper male, three Red Maasai males and two Red Maasai females). The worm counts (PCVs) for these lambs were 180(28), 180(24), 2330(14), 470(17) and 1520(17). For technical reasons no samples were available from one of the Red Maasai females. The Dorper male died of pneumonia. Observations of weakness before death, generalised oedema and low PCVs associated with worm counts of over 1000 suggested that six of the 11 Red Maasai lambs died of haemonchosis. The carcasses of the other three Red Maasai lambs were pale with scanty perirenal, pericardial and omental fat and though the PCVs were not below 20, the lambs had some hydropericardium and some ascites. Some whitish foci probably of necrosis together with petechiae and oedema of the abomasal mucosa were other common features in these animals.

Faecal egg counts

Patency was established between Day 15 and Day 23 after the first infection for both breeds. Two peaks of mean faecal egg counts occurred between Weeks 3 and 4 and 8 and 9 post infection and these were higher in the Red Maasai than in the Dorper (Fig.7.1a). An apparent self-cure occurred in both breeds after the second infection, with egg counts falling rapidly from 5000 and 8000 to 100 and 400 in the Dorper and Red Maasai respectively in two weeks. Following the second infection there was a different pattern of faecal egg output in the two breeds. While the Red Maasai had higher and more sustained peaks compared with those seen after the primary infection, the Dorper had a reduced faecal egg output. After the third infection in Week 17, there was no apparent self-cure and although there were fluctuations, no marked increases in egg output occurred in either breed. Towards the end of the experiment the mean egg outputs in both breeds converged.

Statistical analysis showed a highly significant breed difference between the Dorpers and the Red Maasai (Table 7.1) with the Dorpers having lower counts. The

breeds also responded differently to the three infections and individual animal variation was marked.

PCVs

The mean PCVs (Fig.7.1b) were higher initially in the Red Maasai and remained so until around the time of the second infection when the trend reversed; the Dorpers then had slightly higher PCVs until the end of the experiment. The mean pre-infection levels were generally above 30% in both breeds but after the first infection there was a decline in mean PCV up to the third week when values started showing a steady rise. Another steady decline in mean PCV which was more marked in the Red Maasai was observed nearly four weeks after the second infection. Towards the end of the experiment the values in both breeds had almost returned to pre-infection levels.

Differences between breeds were highly significant (Table 7.2), individual animal variation was marked and the mean values differed significantly between the three infections.

Peripheral eosinophil counts

Although high prior to the first infection, eosinophil counts (Fig.7.2) were similar in both breeds and there appeared to be little obvious response to either primary or secondary infection. Following the third infection, however, both breeds showed increases in eosinophil counts which were generally higher in the Dorpers.

There were very significant breed differences in eosinophil counts and individual variation and breed by infection interaction were highly significant (Table 7.3).

Faecal egg counts between the sexes of both breeds

A comparison of the changes in mean faecal egg output between the sexes of both

breeds is shown in Fig. 7.3. While Dorper males appeared to excrete more eggs than Dorper females the egg excretion in Red Maasai males and females was similar.

Total abomasal worm counts and worm lengths

The mean total worm counts in the abomasa including the counts in animals which died during the experiment were 409 (range 0-2470) and 815 (10-2450) for the Dorper and Red Maasai respectively. Analysis of variance showed a significant ($p< 0.05$) difference between the breeds. Taking only the animals which survived to the end of the experiment the mean worm counts were 421 (0-2470) and 328 (10-940) for Dorper and Red Maasai and the difference in this case was not significant ($p<0.63$). The distribution of total worm counts including or excluding counts in lambs which died before the end of the experiment is shown in Fig.7.4a. From the graph it is seen that while all the Red Maasai lambs that died had more than 1000 worms, a lone Dorper which survived to the end of the experiment had over 2000 worms. The difference in worm burdens between Dorper females and males was significant ($p<0.04$) but not between Red Maasai females and males ($p<0.41$).

Differences in worm lengths of parasites recovered at necropsy were significant between the breeds ($p<0.02$ for females and $p<0.01$ for male worms), the worms from the Red Maasai being larger. Individual animal variations were also significant (Appendix 6).

No larvae were found in digests of the abomasal mucosa from any of the lambs.

ELISA O.D.s

With the adult worm and larval antigens differences in ELISA O.D. values obtained on the seven occasions were not significant between the breeds. There was a response to the two antigens following the primary infection with O.D. values rising above 0.15 (Fig.7.5). The other two infections did not elicit any obvious further

responses.

Analyses of variance on O.D.s with larval antigen showed that only individual animal variations were significant (Table 7.4). With adult worm antigen, however, there was a significant effect of the time of sampling in addition to the highly significant individual variations.

7.3.2 Group 2: six month old lambs

Haemoglobin types

All the lambs in this group were of haemoglobin type AA.

Mortalities

During the first infection period one Dorper and three Red Maasai, all males died. Their worm burdens (last PCVs) were 610(23), 1210(21), 300(17) and 400(20). In the second infection period three Red Maasai males died and at necropsy their worm burdens and PCVs were 1100(13), 2710(14) and 2460(8). A further two Red Maasai lambs, one female and one male, died after the third infection. Their worm burdens (PCVs) were 2460(9) and 2380(11). From the evidence of low PCVs, generalised oedema and total worm counts five Red Maasai lambs were presumed to have died of haemonchosis. The carcasses of the remaining lambs were pale with scanty perirenal, pericardial and omental fat and though the PCVs were not below 20 the lambs had some hydropericardium and some ascites.

Faecal egg counts

In the Dorper lambs of Group 2 the egg counts rose to a peak of 6400 epg six weeks after the first infection and showed a general decline thereafter (Fig.7.6a). Two smaller peaks of 4400 and 4800 epg occurred two weeks and five weeks after the second infection. Following the third infection in Week 17 egg output declined to

below 2000 epg in two weeks but thereafter the egg output increased and fluctuated between 2000 and 4000 epg. After the first infection in the Red Maasai, egg counts rose to peak at 7800 epg in Week 6 and again, just prior to the second infection, to a slightly higher peak of 8500 epg. After the second infection the mean egg count remained high and fluctuated between 6000 and 10,000 epg. As with Dorpers, egg counts fell considerably within two weeks of the third infection from 6000 to 2900 epg. Subsequently the counts varied between 3000 and 7000 epg.

Again Dorpers had significantly lower egg counts than the Red Maasai (Table 7.1). Individual animal variation and the interaction of breed by infection were highly significant.

PCVs

The PCVs showed a similar pattern in both breeds. They fell gradually to reach their lowest values in Week 9 (Fig.7.6b) and then showed a general rise with values fluctuating between 24 and 29% in both breeds. Initially the Red Maasai had slightly higher mean PCVs than the Dorpers but after Week 3 they were generally slightly lower.

The Dorpers had the higher values but no significant difference occurred between the two breeds (Table 7.2). Individual animal variations and the interaction of breed by infection were however, highly significant.

Peripheral eosinophil counts

Changes in eosinophil counts also showed a similar pattern in both breeds (Fig.7.7). There was little apparent response to the primary infection but increases in the counts were seen after the second and third infections. The values were generally higher in the Dorpers. In both breeds the third infection was followed by increased eosinophil responses at one, six and nine weeks post infection before the counts

declined to pre-infection levels.

Significant differences (Table 7.3) occurred between the two breeds with the Dorpers again showing higher counts than the Red Maasai. A significant breed by infection interaction also occurred and individual animal variations were highly significant.

Faecal egg counts between the sexes of both breeds

The changes in mean faecal egg counts between the sexes of both breeds are illustrated in Figure 7.8. As with the Group 1 lambs, the female and male Red Maasai appeared to excrete similar numbers of nematode eggs while the Dorper males had a higher egg output than the females.

Total abomasal worm counts and worm lengths

The mean abomasal total worm counts including the counts of animals which died during the experiment were 439 (range 0-3920) and 683 (40-2710) for the Dorper and Red Maasai respectively. Analysis of variance showed a significant ($p<0.05$) difference between the breeds. Taking only those animals that survived to the end of the experiment the mean total worm counts were 430 (0-3920) and 323 (40-1410) for Dorper and Red Maasai and the difference was not significant ($p<0.23$). The mean counts were significantly ($p<0.001$) lower in Dorper females than males and Red Maasai females also had a significantly ($p<0.01$) smaller worm burden than the males. The Red Maasai lambs in Group 2 which died before the end of the experiment had at least 1000 worms and a lone Dorper male survived to the end with over 3000 worms (Fig. 7.4b).

Worm lengths were not significantly different ($p< 0.53$ for female and $p<0.88$ for male worms) between the breeds in this group but within animal variations were highly significant (Appendix 7).

Eggs per adult female worm

The mean number of eggs per adult female worm (± SE.) were 183(22.6) and 180(24.6) for worms recovered in the Dorper and Red Maasai lambs respectively and the ranges were four to 339 and 13 to 437. The distribution of these eggs/female appeared to be bimodal (Fig. 7.9) and a parametric analysis would not have been appropriate. Most female worms in both breeds had between 200 and 300 eggs.

ELISA O.D.s

ELISA results in this group appeared to be inconsistent. With the adult worm antigen, the O.D. values were rising prior to the primary infection in both breeds (Fig.7.10a). After infection the rise in O.D.s continued to a peak then fell. A small rise was observed in the Dorpers after the second infection while there was no response in the Red Maasai. No assays were carried out after the third infection. With larval antigen the Red Maasai appeared to have responded to the primary infection only while the Dorpers appeared to have responded to the secondary infection (Fig.7.10b).

There were no significant breed differences, but individual animal variation was highly significant (Table7.4).

Table 7.1: Analysis of variance on log_{10}(EPG+10) for lambs infected three times with 5000 *H. contortus* L_3 at eight to nine week intervals from four (Group 1) and six (Group 2) months of age

Factor	Degrees of freedom	Mean square	F	Probability>F
Group 1				
Breed	1	21.00	8.6	0.0054
Animal(breed)	41	2.63	5.7	0.0001
Breed*infect.	2	12.56	27.2	0.0001
Time(infect.)	24	20.5	44.4	0.0001
Group 2				
Breed	1	30.00	12.2	0.0009
Animal(breed)	49	4.21	15.4	0.0001
Breed*infect.	2	7.23	26.5	0.0001
Time(infect.)	28	15.77	57.9	0.0001

Model: Parameter= breed animal(breed) breed*infection time(infection)

Parentheses indicate animal nested within breed and time nested within infection and * denotes breed by time interaction

Table 7.2: Analysis of variance on PCVs for lambs infected three times with 5000 *H. contortus* L3 at eight to nine week intervals from four (Group 1) and six (Group 2) months of age

Factor	Degrees of freedom	Mean square	F	Probability>F
Group 1				
Breed	1	218.28	13.92	0.0002
Animal(breed)	41	105.53	6.73	0.0001
Breed*infect.	2	375.00	23.91	0.0001
Time(infect.)	21	92.84	5.92	0.0001
Group 2				
Breed	1	585.52	3.76	0.0580
Animal(breed)	48	232.86	18.49	0.0001
Breed*infect.	2	132.54	10.52	0.0001
Time(infect.)	28	99.77	7.92	0.0001

Model: Parameter= breed animal(breed) breed*infection time(infection)

Parentheses indicate animal nested within breed and time nested within infection and * denotes breed by time interaction

Table 7.3: Analysis of variance on $\log_{10}$(eosinophil count+1) for lambs infected three times with 5000 *H. contortus* L_3 at eight to nine week intervals from four (Group 1) and six (Group 2) months of age

Factor	Degrees of freedom	Mean square	F	Probability>F
Group 1				
Breed	1	0.02	7.78	0.005
Animal(breed)	41	0.005	2.52	0.0001
Breed*infect.	2	0.02	9.21	0.0001
Time(infect.)	22	0.004	1.89	0.008
Group 2				
Breed	1	0.03	4.93	0.0300
Animal(breed)	49	0.01	5.27	0.0001
Breed*infect.	2	0.006	3.05	0.0480
Time(infect.)	28	0.01	5.52	0.0001

Model: Parameter= breed animal(breed) breed*infection time(infection)

Parentheses indicate animal nested within breed and time nested within infection and * denotes breed by time interaction

Table 7.4: Analysis of variance on log_{10}(O.D.s) for lambs infected three times with 5000 *H. contortus* L_3 at eight to nine week intervals from four (Group 1) and six (Group 2) months of age

Factor	L_3 antigen		Adult worm antigen	
	DF	Probability	DF	Probability
Group 1				
Time	4	0.7952	4	0.0370
Breed	1	0.9165	1	0.3174
Animal(breed)	36	0.0001	36	0.0001
Time*breed	4	0.8460	4	0.1088
Group 2				
Time	4	0.5455	4	0.1056
Breed	1	0.4926	1	0.3818
Animal(breed)	39	0.0001	44	0.0001
Time*breed	4	0.0662	4	0.2563

Model: Parameter= breed animal(breed) time time*breed

Parentheses indicate animal nested within breed and time nested within infection and * denotes breed by time interaction. DF= degrees of freedom

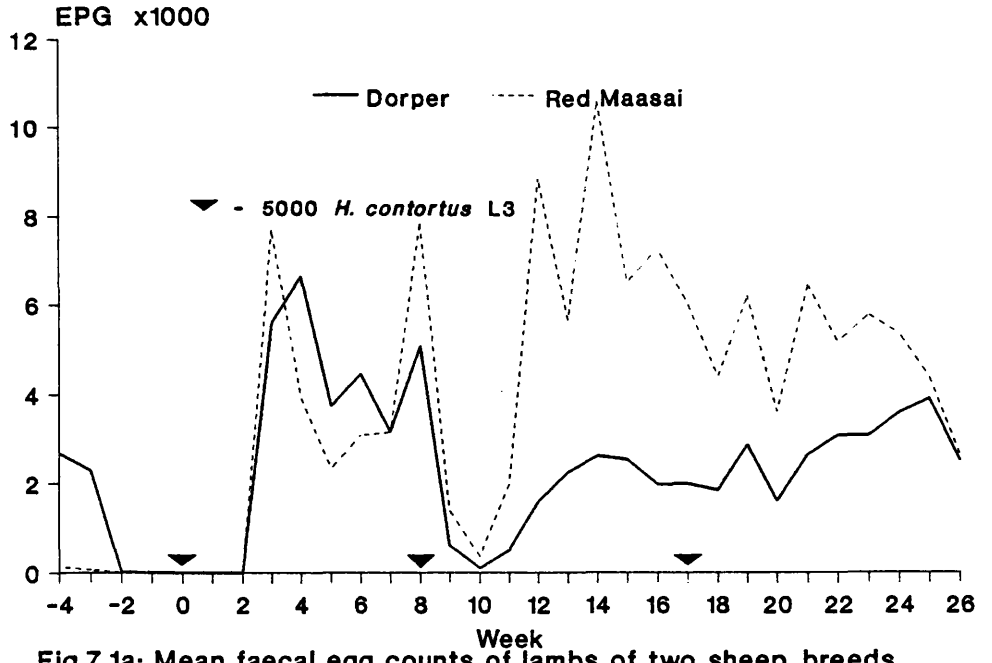

Fig.7.1a: Mean faecal egg counts of lambs of two sheep breeds infected with three doses of *H. contortus* L3 from four months of age

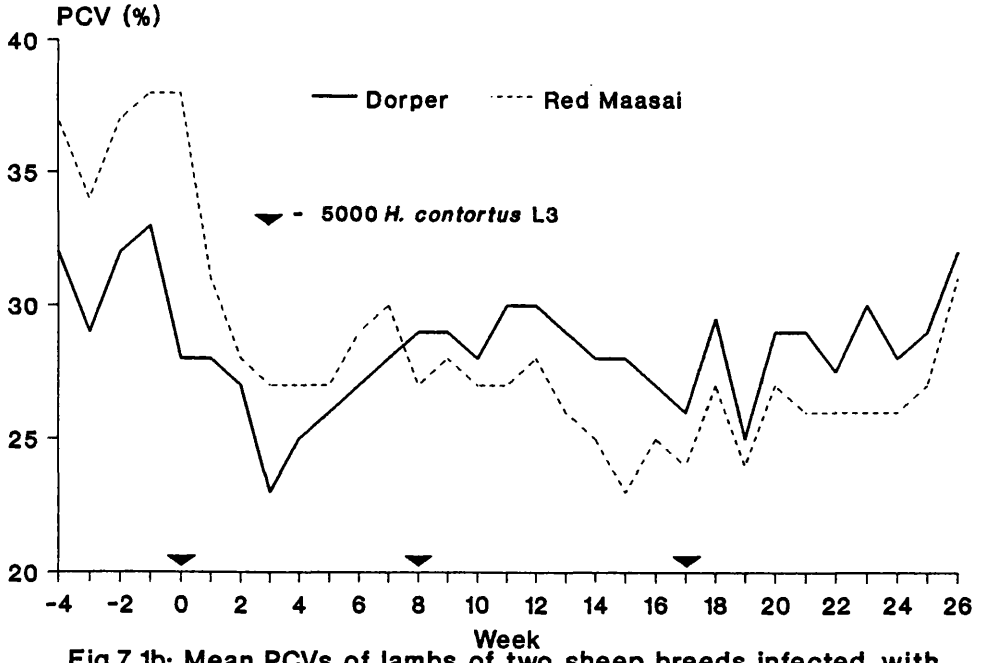

Fig.7.1b: Mean PCVs of lambs of two sheep breeds infected with three doses of *H. contortus* L3 from four months of age

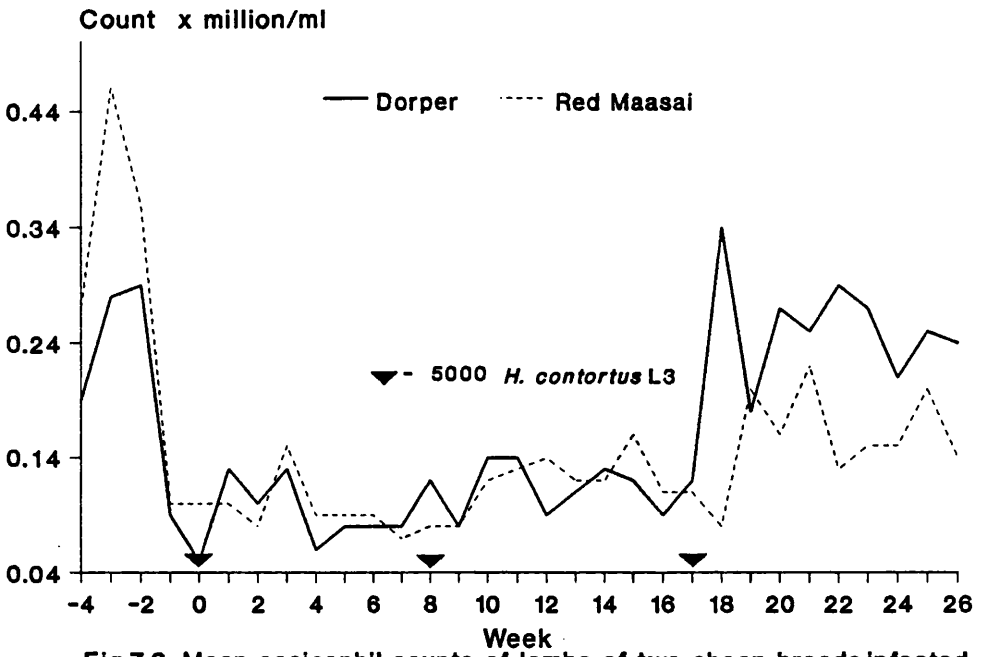

Fig.7.2: Mean eosinophil counts of lambs of two sheep breeds infected with three doses of *H. contortus* L3 from four months of age

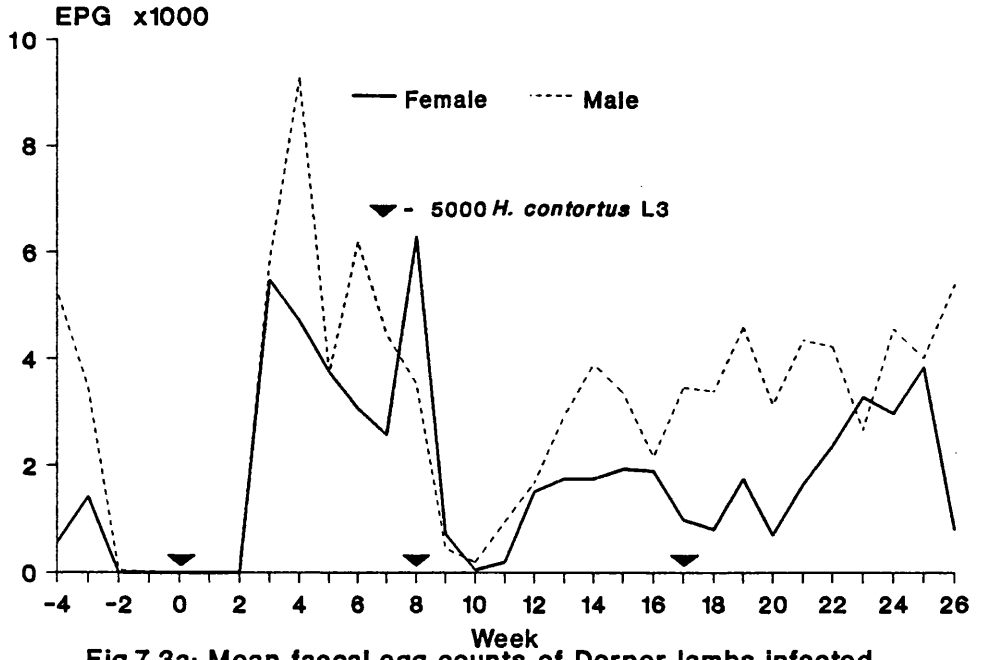

Fig.7.3a: Mean faecal egg counts of Dorper lambs infected with three doses of *H. contortus* L3 from four months of age

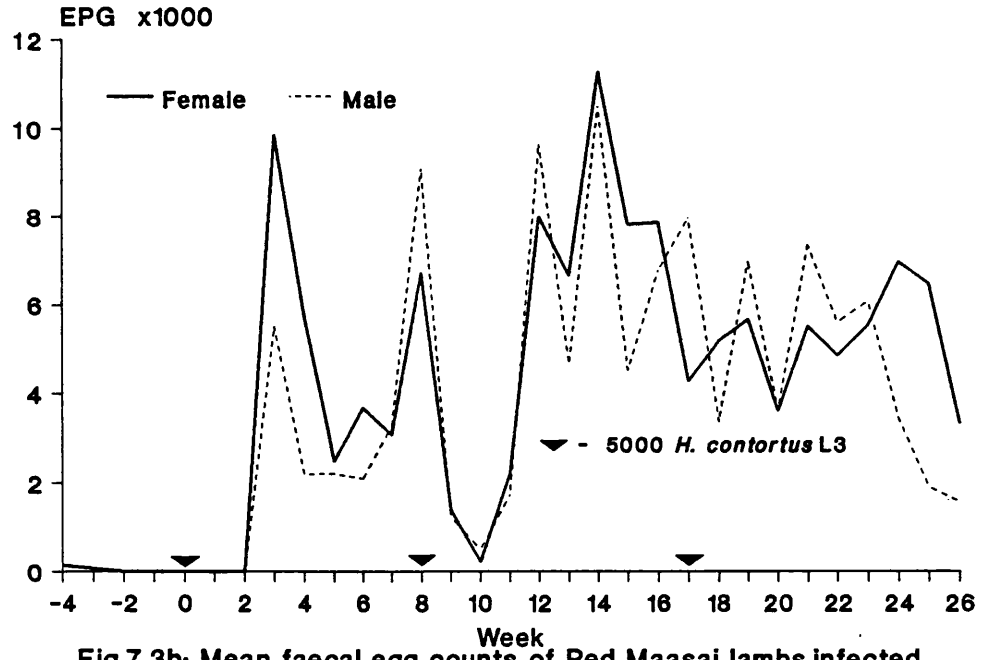

Fig.7.3b: Mean faecal egg counts of Red Maasai lambs infected with three doses of *H. contortus* L3 from four months of age

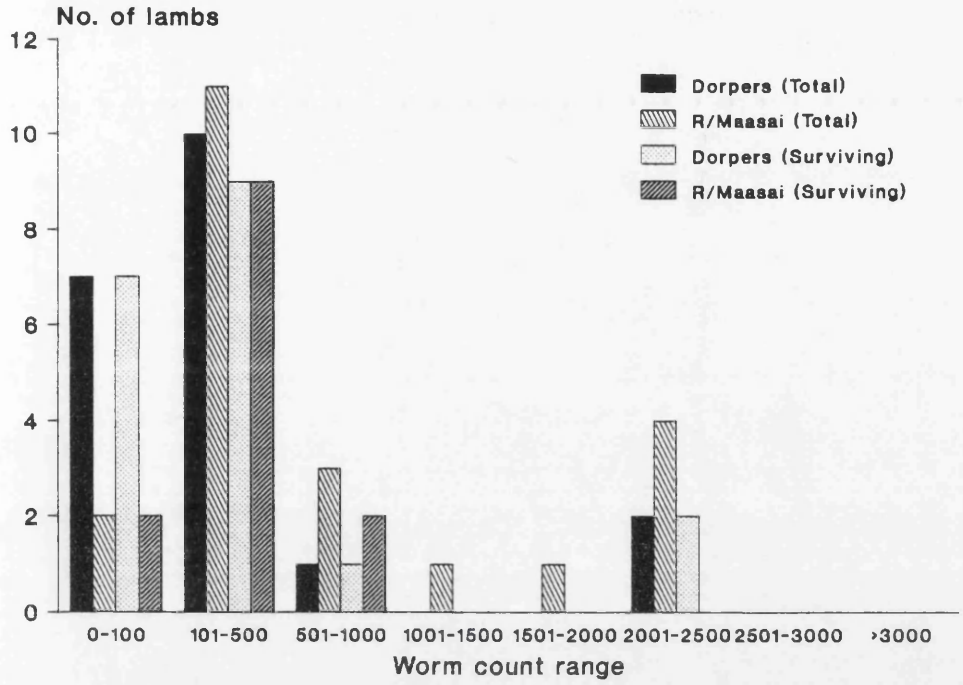

Fig.7.4a: Distribution of *H. contortus* worm counts in Group 1 lambs

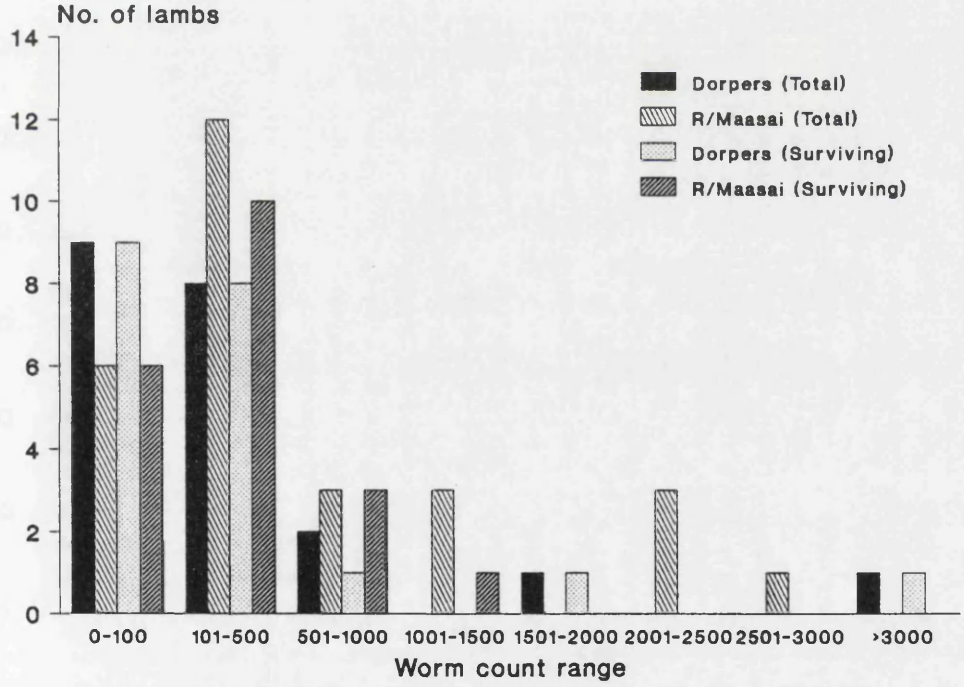

Fig.7.4b: Distribution of *H. contortus* worm counts in Group 2 lambs

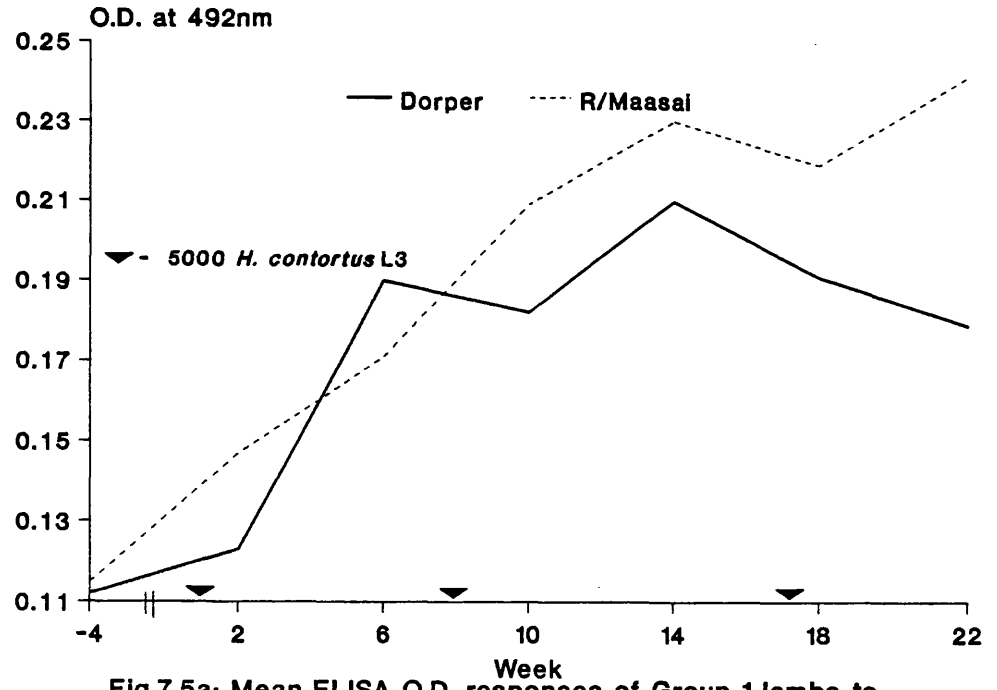

Fig.7.5a: Mean ELISA O.D. responses of Group 1 lambs to *H. contortus* adult worm antigens after three infections

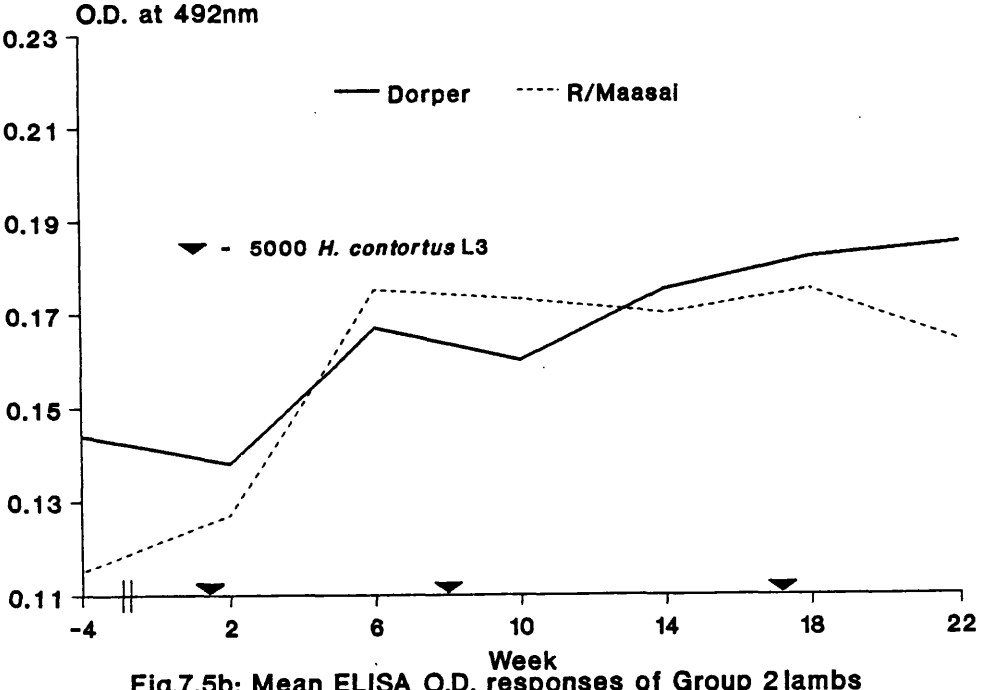

Fig.7.5b: Mean ELISA O.D. responses of Group 2 lambs to *H. contortus* L3 antigens after three infections

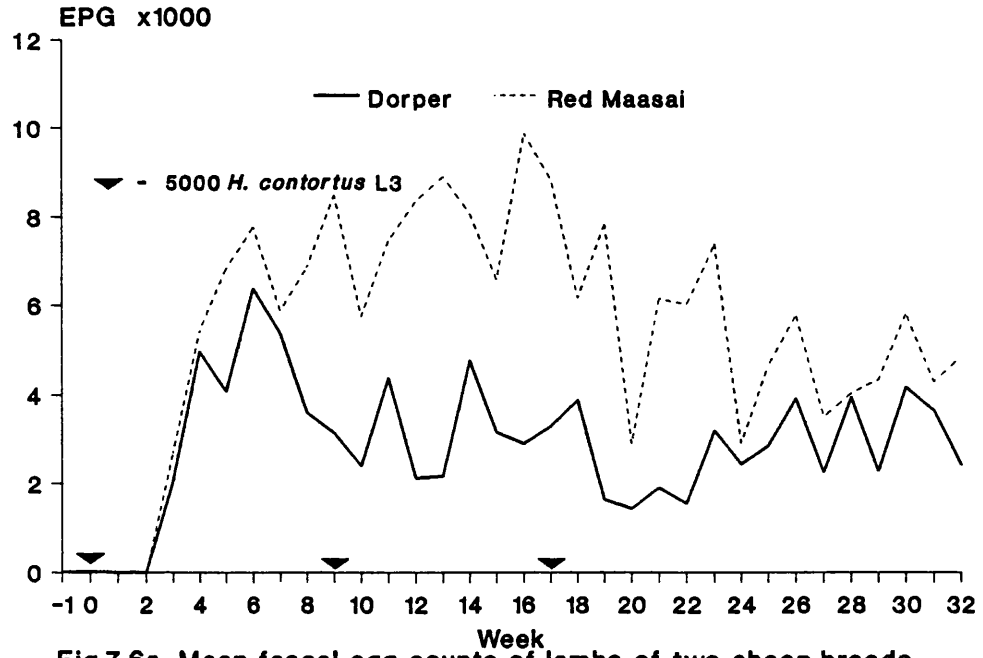

Fig.7.6a: Mean faecal egg counts of lambs of two sheep breeds infected with three doses of *H. contortus* L3 from six months of age

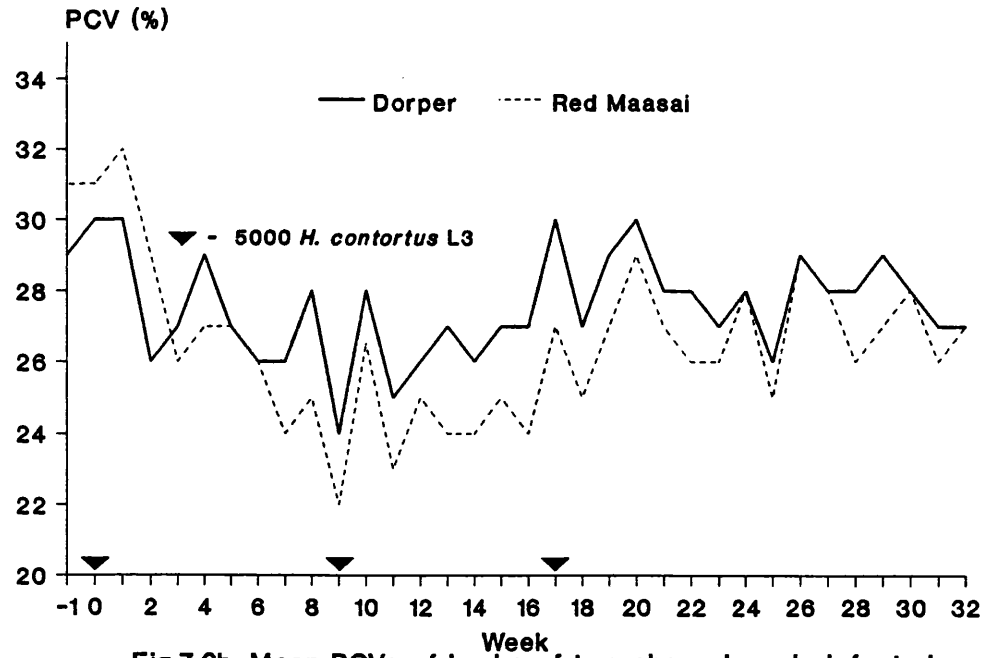

Fig.7.6b: Mean PCVs of lambs of two sheep breeds infected with three doses of *H. contortus* L3 from six months of age

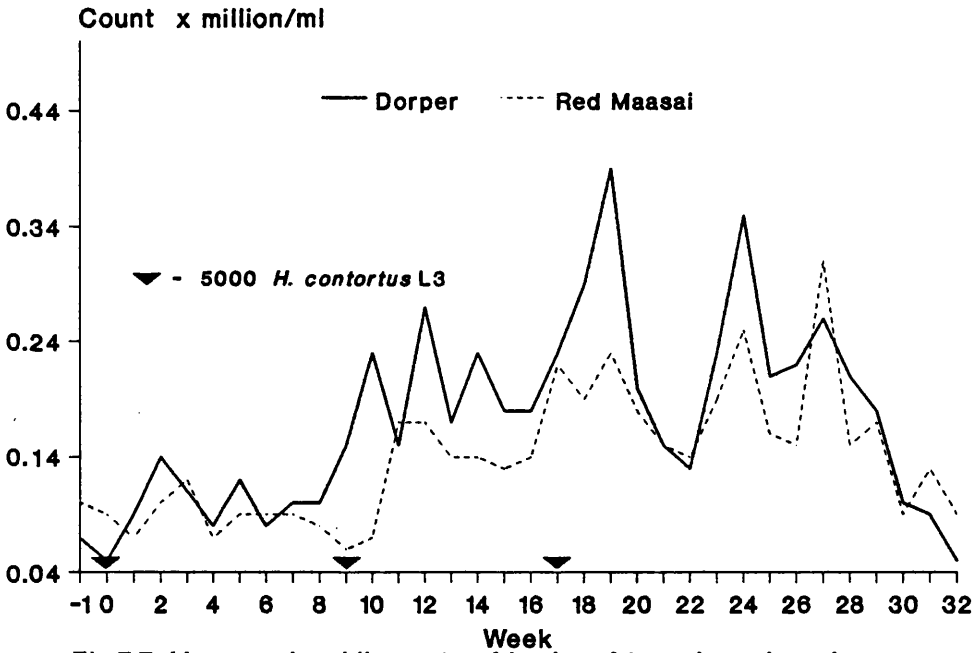

Fig.7.7: Mean eosinophil counts of lambs of two sheep breeds infected with three doses of *H. contortus* L3 from six months of age

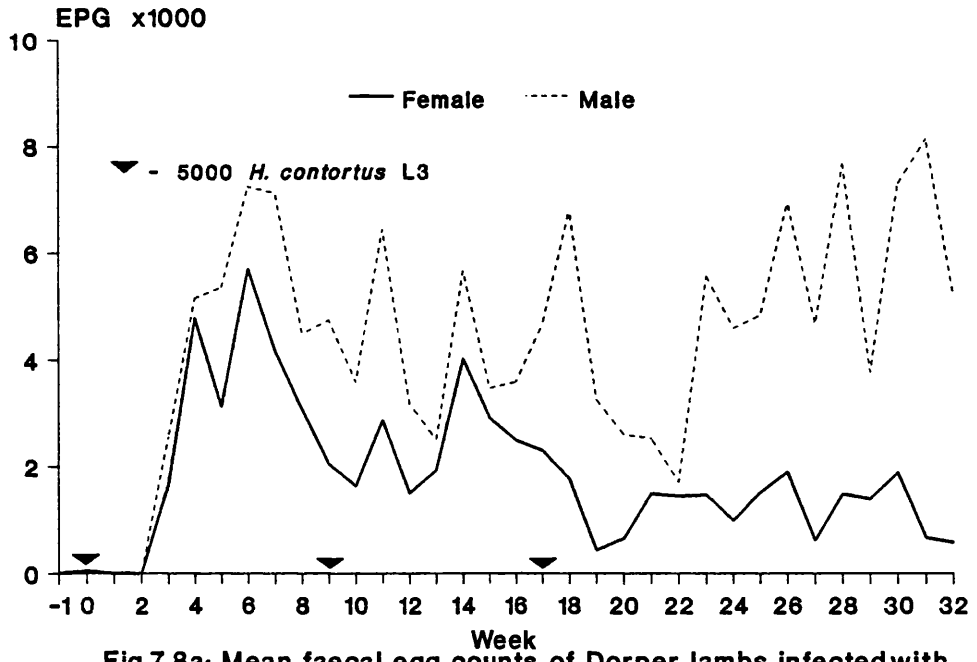

Fig.7.8a: Mean faecal egg counts of Dorper lambs infected with three doses of *H. contortus* L3 from six months of age

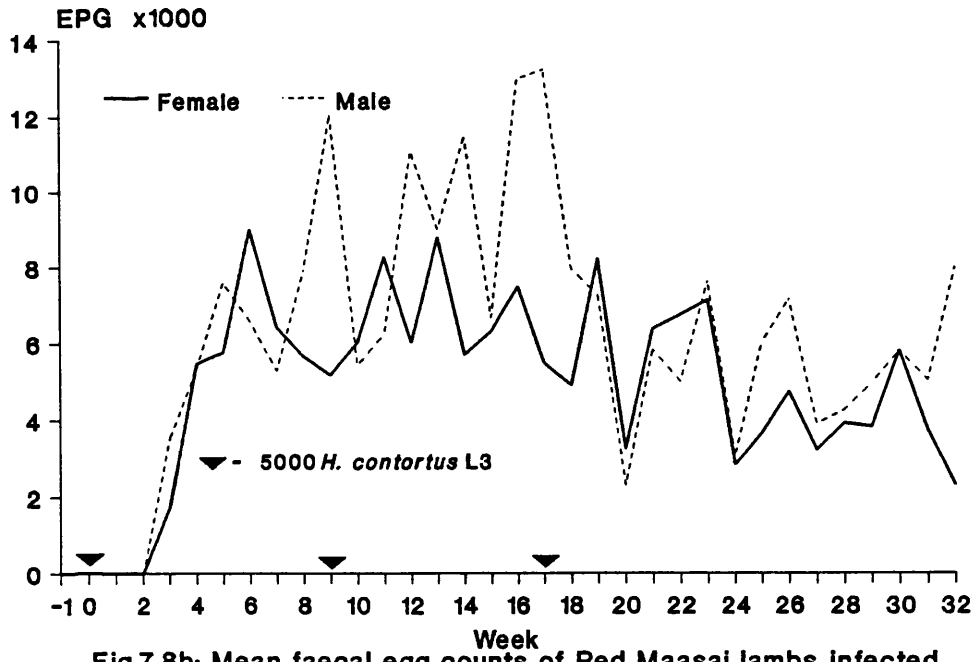

Fig.7.8b: Mean faecal egg counts of Red Maasai lambs infected with three doses of *H. contortus* L3 from six months of age

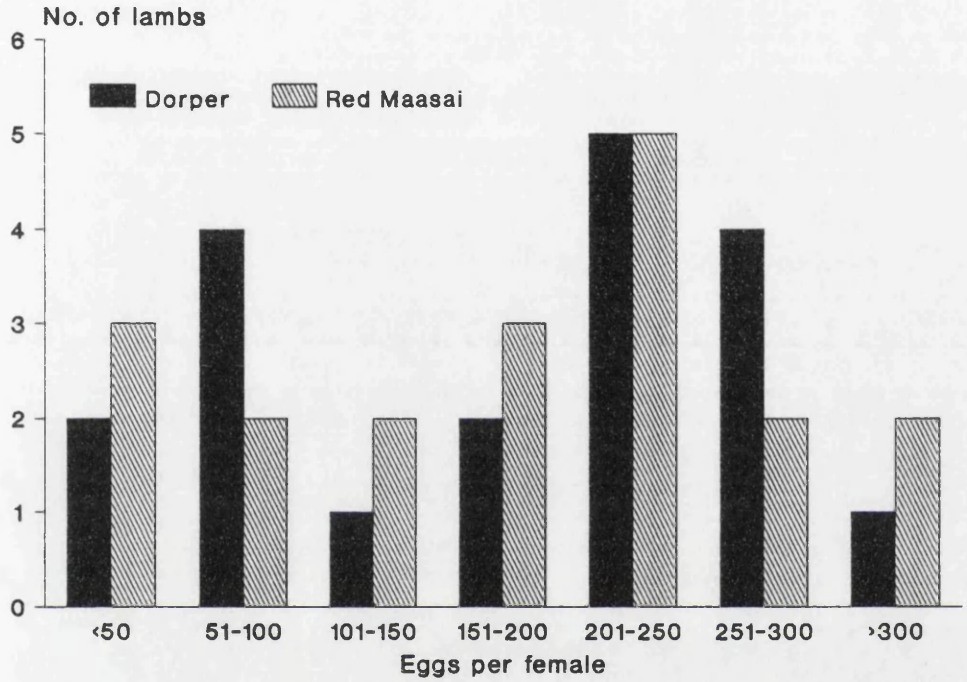

Fig.7.9: Egg distribution per adult *H. contortus* female in Group 2 lambs

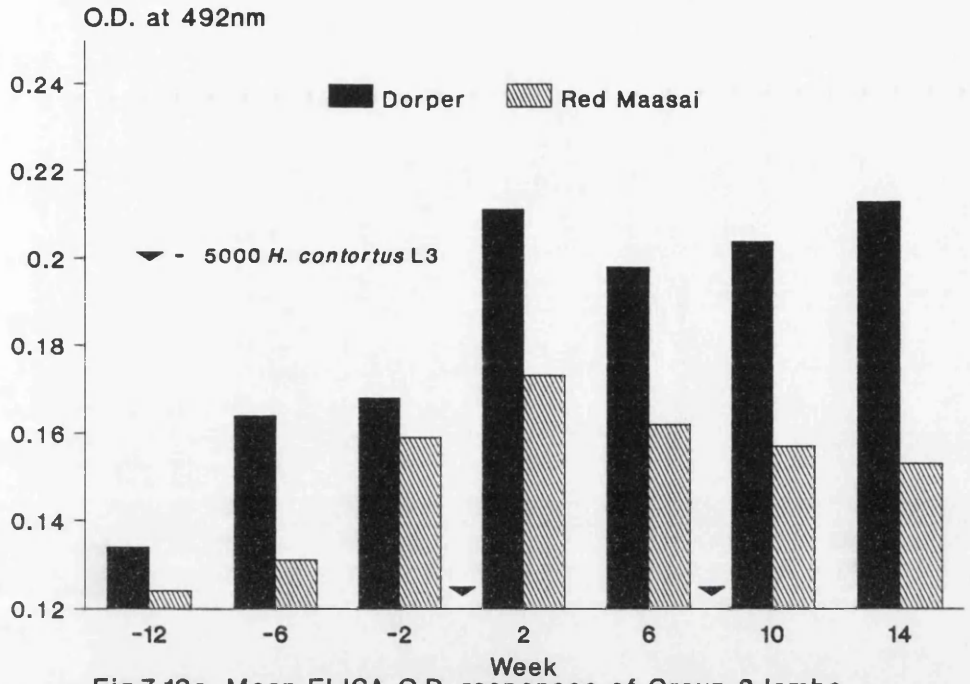

Fig.7.10a: Mean ELISA O.D. responses of Group 2 lambs to adult *H. contortus* antigens after two infections

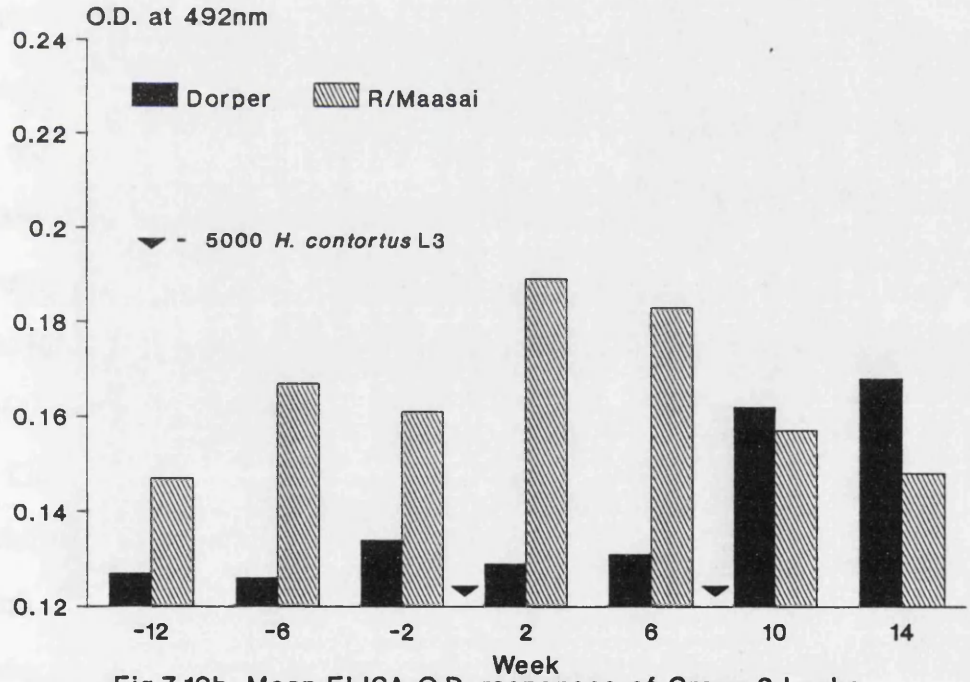

Fig.7.10b: Mean ELISA O.D. responses of Group 2 lambs to *H. contortus* L3 antigens after two infections

7.4 DISCUSSION

The results of this experiment were in marked contrast to those obtained in three previous studies (Chapters 3 to 5) in which the Red Maasai consistently appeared to be more resistant to *H. contortus* than the Dorper on the basis of lower faecal egg output and mortality following artificial and/or natural infection. They are, however less contrasting with those of Chapter 6 where no differences were observed between the two breeds following the same regime of infections. The results are also at variance with other published reports in which the Red Maasai has shown superior resistance to haemonchosis compared with several other breeds (Allonby 1975; Castellino 1976; Preston and Allonby 1978, 1979a). The only notable differences between this experiment and previous studies is that young lambs whose ages were accurately known were used and were reared up to three months worm free.

We can only speculate on the reasons for this anomalous situation of apparent reversal in resistance to *H. contortus* infection between the two breeds. First, it may be that the superior resistance seen in adult Red Maasai depends on exposure to a heavy infection or repeated infection early in life. Although these lambs were not completely worm-free before artificial infection the level of infection was low in the Red Maasai in which an average faecal egg count of 120 epg was recorded before treatment. At the same time counts in the Dorpers were 25 and 5 times higher in Groups 1 and 2 respectively. Perhaps the level of prior exposure in the Dorper resulted in the apparently superior response to experimental infection compared with the Red Maasai. However, there is some evidence that early exposure can induce immunological tolerance especially in *H. contortus* endemic areas like Kenya (Lopez and Urquhart 1967; Allonby, 1975). Allonby (1975) reared a group of Merino lambs on a haemonchosis-endemic pasture. The mean faecal egg count reached 7000 epg when the lambs were only two months old. During this period a

few of the lambs died. In another group of lambs which were exposed to a much lower level of infection, egg counts reached a mean of only 600 at five months of age. When the two groups of lambs were subsequently exposed to the same natural challenge for 20 weeks those lambs that had experienced a heavy infection early attained up to 4700 epg while the other group had a maximum egg count of only 500 epg in the first 14 weeks. Subsequently the counts in both groups were similar. It thus appeared that a relatively heavy early exposure had a negative influence on later immunity. It is possible that in the present study, the Dorpers were not exposed early enough for the induction of tolerance to occur. However, the results of Benitez-Usher *et al.* (1977) did not support the findings of induction of tolerance. In their study, the effect of administering six monthly doses of 100-200 normal larvae to 10 week old Scottish Blackface lambs on the response to vaccination with irradiated larvae in later life was investigated. The worm reductions after challenge in infected lambs which received regular anthelmintic treatment between infections and in those that did not, were 46% and 88% respectively. These reductions were not statistically different from the 95% reduction obtained in vaccinated and challenge controls. However, in this study the total number of larvae administered to each lamb was low. Where larger numbers of larvae were administered, that is, six sensitising infections of 3000 L_3, prior to challenge in seven month old Merino-Border Leicester cross-bred lambs, immunity was found to be poor (Dineen and Wagland, 1966). It is possible that there is an age at which exposure is likely to induce tolerance and that the Dorpers were not exposed within that age. However, since even when lambs were infected at six months, i.e. 11 weeks after treatment the Dorper still showed a higher level of resistance than the Red Maasai, early exposure seems untenable as the cause of this reversal of responses.

Second, it may be that the smaller Red Maasai lambs in this study (average dressed weights were 13 and 21kg for Red Maasai and Dorper lambs respectively) were not

getting their fair share of concentrates compared to the larger Dorpers since both breeds were housed together. There is evidence that well-fed animals can resist parasitism better than those less adequately fed (Whitlock 1949). It has been shown that lower PCVs and higher egg counts occur in poorly fed compared with well nourished *H. contortus*-infected sheep though there may be no difference in worm counts at necropsy (Courtney *et al.*, 1985a). Florida Native sheep unexpectedly performed poorly compared to Barbados and domestic cross breds on the basis of egg counts in one of their three experiments because the lambs of this breed were repeatedly pushed away from the feed troughs and their wool plucked by animals of the other breeds (Courtney *et al.*, 1985a).

Differences in worm counts between the breeds at necropsy were significant ($p< 0.05$) only when counts in the Red Maasai lambs that died before the end of the experiment were included in the analyses. When these were included the Dorper appeared to be more resistant. This was true in both Group 1 and Group 2 lambs. However, when worm counts of only those lambs that survived to the end of the experiment were analysed there was no difference between the two breeds. This suggests that haemonchosis either alone or in combination with other factors was responsible for the deaths in the Red Maasai. Although the cause of death of the five and the three Red Maasai lambs of Groups 1 and 2 respectively which had worm counts of less than 1000 was not conclusively established, it would appear that the primary cause was haemonchosis possibly in conjunction with inadequate nutrition. Such a situation has been observed by Allonby and Urquhart (1975) in the field in Kenya with Merino ewes. They described a syndrome of chronic haemonchosis in which some ewes died with burdens of 500-600 adult worms without obvious clinical signs. These animals were grazing poor pastures but worm-free ewes on equally poor pastures in an adjacent paddock maintained fairly good body condition. Studies on two year old Merino wethers experimentally infected

with *H. contortus* supported these findings in that while PCVs appeared to be normal, radioisotopic techniques revealed continuous abomasal haemorrhage (Dargie and Allonby, 1975). Progressive depletion of the host's iron reserves ultimately resulted in iron deficiency and at this stage the animal could no longer haemopoietically compensate for further haemorrhage. Another explanation is that some expulsion occurs prior to death. This possibility was advanced by Whitlock (1949, 1955a) to explain the frequent deaths due to haemonchosis without a sufficient number of parasites in the abomasum to account for the observed lesions.

Out of a total of 33 Red Maasai lambs from both groups that survived to the end of the experiment only one lamb had a total count of over 1000 (1410); all the other lambs with counts over 1000 had died earlier. Since Castellino (1976) found that expulsion of experimental infections of *H. contortus* occur from Day 15 in the Red Maasai, it implies that establishment takes place first before the worms are expelled and this has been observed in vaccination trials against nematode infections in laboratory animals (reviewed by Miller, 1986). Further, Preston and Allonby (1978) recovered only 3.5% of an infective dose of *H. contortus* from the Red Maasai four weeks after infection compared to 22.8% in the Hampshire Down. Assuming then that factors such as nutrition were not constraints on the expression of resistance in this study, the observations tend to suggest that failure to expel the worms may result in haemonchosis even with a relatively light worm burden. The findings support the suggestion of Riffkin and Dobson (1979) that sheep which show superior resistance to establishment of *H. contortus* owing to their immunological competence may not necessarily be able to withstand the pathogenic effects of parasites which survive the immune responses. However, since worm establishment rates were not assessed in the present study, it is difficult to validate these two suggestions.

It is not clear why there were significant breed differences in worm lengths in

Group 1 and not in Group 2 yet the relative worm burdens appeared to be similar. The worms were significantly longer in the Red Maasai than in the Dorper in Group 1. Kloosterman et al. (1991) found that male *C. oncophora* were significantly longer in worms recovered from three month old compared to those recovered in older calves. In the present experiment there may therefore have been an age effect on worm lengths which reduced the breed differences with time.

The significant ($p<0.05$) differences in worm counts observed between female and male Dorpers in Group 1 probably implies that this breed reaches sexual maturity much earlier than the Red Maasai. It is perhaps for the same reason that the differences between Dorper female and male lambs of Group 2 were even more significant ($p<0.01$) and why the differences between female and male Red Maasai were also significant ($p<0.01$) in this group. Courtney *et al.* (1985a) found sex differences in egg output between groups of lambs of several breeds only after the lambs reached puberty, when female lambs showed greater resistance than males. In contrast, Windon and Dineen (1981) found greater responses in female than in male lambs vaccinated with irradiated larvae of *T. colubriformis* at two to three months, that is, even before puberty. It has also been suggested that body condition may be more important than chronological age (Abbott *et al.*, 1988).

Preston and Allonby (1978) compared the resistance of the Red Maasai, Hampshire Down, Corriedale and Merino to haemonchosis after an experimental infection. They reported that the superior resistance of the Red Maasai was not likely to be due to differences in the acquisition of larvae from pasture between the Red Maasai and other breeds since there were no observed differences in grazing habits. It is not clear how they arrived at this conclusion but it was presumably due to their finding of breed differences that were consistent with the results of an earlier natural infection study (Preston and Allonby, 1979a). The lambs in the present study inadvertently acquired natural infections which were much higher in

the Dorper than in the Red Maasai and it is interesting that there was an apparent breed difference in the early acquisition of infection.

The self-cure phenomenon, first described by Stoll (1929) and clearly seen during this experiment with Group 1 lambs was the so called 'classical' type reported by Gordon (1948). Extensive studies in Australia (Gordon, 1967) have shown that the response of Merino sheep to re-infection with *H. contortus* can be classified into one of four main categories: loss of existing worm burden with no re-establishment, that is, self-cure and protection; loss of existing infection followed by re-infection, the 'classical' self-cure; temporary suppression of egg production followed by hyperinfection and no loss of existing infection and no establishment of new infection. This classification is based on egg counts and worm burdens and illustrates the wide variation in the response of infected sheep to a new larval challenge (Dargie and Allonby, 1975). Early work indicated that the reaction was immunological, resembling an immediate-type hypersensitivity reaction, the allergen(s) probably originating from the ex-sheathing fluid of larvae after the third ecdysis (Stewart, 1953; Dargie and Allonby, 1975). These types of self-cure can also be induced with experimental infections within the same group of animals (Dargie and Allonby, 1975). IgA antibodies were thought to play a role in self-cure (Mulligan, 1968) and later it was found that abomasal anti- *H. contortus* L_3 specific IgA antibodies were indeed associated with the reaction (Charley-Poulain *et al.*, 1984). In addition to this immunologically defined self-cure, Lopez and Urquhart (1967) observed that self-cure could also be associated with the intake of lush grass.

ELISA results indicated weak serum antibody responses to primary infection in Group 1 lambs and subsequent infections did not stimulate any further detectable responses. Similar observations have been reported in Manchego lambs and Merino ewes (Cuquerella et al., 1991; Adams and Beh, 1981). In contrast Smith and Christie (1978) found increased IgG levels in sheep after each of two vaccination

doses with irradiated *H. contortus* larvae and a further increase after challenge with normal larvae. In Group 2 the results were inconsistent. The results of assays done at monthly intervals may be of limited value compared with those done at shorter intervals. However, in this study the number of samples involved was fairly large and these were screened at longer intervals first so that any obvious differences detected between the breeds could be investigated further by analysing sera drawn at shorter intervals. Since no such differences were observed no further assays were carried out. In general estimations of serum antibody responses to *H. contortus* infections in lambs have given equivocal results. Increased antibody levels have been found in both primary infections and hyperinfections (Smith, 1977b; Neilson and van de Walle, 1987), in vaccination trials with irradiated larvae (Smith and Christie, 1978; Smith and Angus 1980) and with injections of *H. contortus* products (Smith 1977a; Smith and Angus, 1980; Munn *et al.*, 1987 ; Neilson and van de Walle, 1987). It is only in a few studies, for example, those by Smith and Christie (1978), Gill (1991) and Gill *et al.* (1993) that an association between serum IgG levels and resistance to *H. contortus* following oral exposure to larvae has been reported. However, this association is stronger with mucosal IgA antibodies (Gill, 1991). In contrast, Charley-Poulain *et al.* (1984) did not find differences between ELISA O.D. values of pre- and post- infection sera. In *H. contortus* infections in sheep it would also appear that the age of the animal is important in determining the serum antibody responses to oral vaccinations. In this regard Duncan *et al.* (1978) could not find any difference in serum IgG levels between control and irradiated-larvae vaccinated two month old lambs while significant increases were observed with similarly vaccinated adult sheep. Immunological memory after challenge infections is sometimes also apparent (Smith and Christie, 1978) while in other cases it is not (Smith 1977b).

In the present experiment an increased peripheral eosinophil response was marked

only after the third infection in the Group 1 animals and after the second infection in the Group 2 animals. This may be related to the development of resistance with both prior infection and increasing age. There is a large body of evidence, especially in laboratory animals, indicating that eosinophils are associated with resistance to helminths and in sheep an inverse relationship between peripheral eosinophilia and worm burdens in *T. colubriformis* infections has been reported (Dawkins *et al.*, 1989; Windon, 1991; Buddle *et al.*, 1992). Similarly, Bradley et al. (1973) recovered fewer adult and larval *H. contortus* worms from Florida Native lambs which had a large proportion of eosinophils in the cellular infiltration of the abomasal mucosa. Gill (1991) also reported significantly more mucosal but not blood eosinophil numbers in resistant than in random-bred Merino lambs after challenge infection. In contrast, Smith and Angus (1980) did not find any differences in eosinophil infiltration in the abomasal mucosa between *H. contortus* irradiated larvae-vaccinated resistant and susceptible Suffolk-Greyface cross lambs. Results of the statistical analyses of the present data show that the eosinophil response was significantly higher in the Dorpers than in the Red Maasai which implies a role for these cells in resistance.

CHAPTER EIGHT

GENERAL DISCUSSION

The experiments described in this thesis were designed to confirm earlier reports that the Red Maasai sheep breed is more resistant to haemonchosis than other breeds. In the first study the resistance of 10-15 month old wethers of four breeds of sheep namely Red Maasai, Romney Marsh, Dorper and Blackhead Somali grazing separate paddocks were compared for one year under conditions of natural exposure. After the end of this period, surviving sheep were treated with an anthelmintic, experimentally infected with 10,000 L_3 and studied for a further eight weeks indoors. The results showed that the Red Maasai were more resistant than the other breeds on the basis of faecal egg counts, PCVs and mortality rates. None of the 15 Red Maasai wethers died during the natural exposure phase while nine Romney Marsh, two Dorper and two Blackhead Somali wethers died of haemonchosis during this period. None of the sheep died of the disease following the artificial infection, presumably because of increasing age and acquired resistance to the disease after the prolonged field exposure. At necropsy, differences in total worm counts between the breeds were not significant but differences in faecal egg counts and PCVs were highly significant with the Red Maasai having lower counts and higher PCVs than the other breeds. The lower egg output of the Red Maasai suggested that one target of the immune response was the fecundity of adult female worms but this could also have been influenced by differences in total faecal output. However, it has been suggested that the Red Maasai's resistance depends on its ability to expel the worms from Day 15 of infection (Castellino, 1976) and a study by Preston and Allonby (1978) tended to support this finding. Also, differences in blood loss into the gut between infected Scottish Blackface and Finn Dorset sheep were evident from Day 12 after infection, suggesting that differences in final establishment

depended on the effectiveness of the expulsion mechanism during the time when the worms were developing to sexual maturity (Altaif and Dargie, 1978a).

One notable beneficial effect of the reduced egg output by the Red Maasai wethers is a decreased potential for pasture contamination with infective larvae. This can be of considerable epidemiological importance as shown by Gibson and Everett (1977) when they found substantial differences in *O. circumcincta* L_3 populations between a paddock grazed by resistant sheep and another paddock grazed by a susceptible group.

The higher PCVs of the Red Maasai might suggest that the other breeds lost a significant portion of their worm burdens later than the Red Maasai but no correlation between PCV changes and worm burdens was possible since no necropsies were carried out early to assess establishment rates. However, there were no obvious decreases in egg counts towards the end of the experimental infection period to indicate that this was a possibility. Although blood loss and worm burdens are reportedly well correlated (Altaif and Dargie, 1978a), differences in PCVs due to differences in haemopoietic capacities of infected sheep have also been reported (Dargie and Allonby, 1975).

Serum IgG responses to helminth infection generally correlate poorly with resistance, especially when crude antigens are used, but they have been found to be useful indicators of resistance in *T. colubriformis*-resistant/susceptible lines (Windon and Dineen, 1981) and to correlate significantly and positively to resistance in *H. contortus*- resistant lines of Merino sheep (Gill, 1991). In the present study there were highly significant ($p< 0.0001$) differences in serum IgG ELISA responses, with the Red Maasai having the higher values and this indicated that IgG might play some role in resistance although the general view is that local IgA responses are more important.

The Dorper and the Blackhead Somali sheep behaved similarly particularly with regard to faecal egg output and PCV changes. This is probably due to the genetic relationship between these two breeds. Although the Dorper was

developed from the Dorset and the Blackhead Somali and is now considered a distinct breed, it is possible that in the tropical environment in which it was developed, natural selection has been directed towards *Haemonchus* resistance, making it respond more like a Blackhead Somali. These two breeds appeared to differ only in their serum IgG responses but it has been shown that strains of mice infected with *S. mansoni* may have similar responses in terms of egg output, accumulation of the eggs in tissues and splenomegaly and yet have different antibody responses (Jones and Kusel, 1985).

In the second experiment Red Maasai and Dorper ewes were kept indoors, infected artificially with 5,000 *H. contortus* L_3 and the infection terminated nine weeks later before the ewes were allowed to acquire new infection by grazing together in a naturally contaminated paddock. The Red Maasai was again more resistant as assessed by faecal egg counts. Differences in PCVs, eosinophil counts, serum IgG levels and total serum proteins were less clear-cut but they all tended to support the results of faecal egg counts. These differences were less marked probably because the dose of 5000 L_3 administered during the indoor study was inadequate for adult ewes to show differences in resistance, while in the outdoor study, the Dorper ewes were less exposed when grazing together with the Red Maasai than if they had been grazing a separate paddock. Cumulative egg counts indicated that the Dorpers were contaminating the pastures at twice the level of the Red Maasai and during a periparturient rise in faecal egg counts, which was observed in both breeds, this differential contamination rate was maintained. However, in the absence of non-pregnant ewes the real magnitude of the periparturient rise could not be determined. Although the ewes were not necropsied because they were kept for subsequent breeding, there was evidence once more that the Red Maasai's immune response was directed against fecundity of female worms since no consistent differences in PCVs, total serum proteins, IgG levels and eosinophil counts were observed.

In the third experiment, the effect of trickle infections of nine doses of 1000 *H.*

contortus L_3 at one to two week intervals in four month old 17 lambs each of Red Maasai and Dorper was assessed on the basis of faecal egg counts, PCVs and mortality rates. As in the previous two experiments where natural exposure was alternated with a single experimental infection, the Red Maasai lambs were more resistant than the Dorper lambs. Five Dorpers died of haemonchosis compared with one Red Maasai. Eosinophil responses were significantly higher in infected Red Maasai lambs than in infected Dorper lambs while there were no differences between the two uninfected control groups. This provided stronger evidence than the second experiment that eosinophilia and resistance were positively associated. Depression of total serum proteins was more evident in the infected Dorper than in the infected Red Maasai lambs but there was no difference between the uninfected controls. Hypoproteinaemia is a common sequel of helminth infections, the loss of protein occurring through loss of blood, exfoliated epithelial cells and mucus (Dargie, 1982a; Holmes, 1986, 1987) and is therefore a useful indicator of the effects of infection. These lambs were on a high plane of nutrition and there were no differences in weight gains between the infected groups or between infected lambs and their controls. The dry matter feed intake was, however, higher in each infected group compared with its uninfected control group implying that the infected lambs were not utilising all their feed for growth.

In the fourth experiment six month old Dorper and Red Maasai lambs were exposed to three successive infections with 5000 *H. contortus* L_3 at eight to nine week intervals. No significant parasitological, haematological, biochemical or serological differences were observed between the two breeds. It appears that with good nutrition differences between Red Maasai and Dorper lambs occur in *H. contortus* trickle infections acquired either naturally or experimentally but there may be no differences shown after single infections given at longer intervals. It also appears that trickle infections rather than single large infections or a few large infections given at intervals of several months may be better in

defining comparative resistance status and it has been suggested that the responses of sheep to extended periods of *H. contortus* infection are likely to reflect the operation of a wider range of mechanisms than those operating in abbreviated infections (Barger and Dash, 1987). Also, acquired resistance to *Haemonchus* infections wanes in six to 12 weeks in the absence of re-infection (Jackson *et al.*, 1988) and immunological memory may be poor (Smith, 1977b). In addition, immunologically active components appear to be present in larval and not in adult stages (Silverman, 1970) and therefore continuous larval intake is likely to be more useful than single infections in the expression of resistance.

In the final experiment, it appeared that Dorper lambs were more resistant than Red Maasai lambs on the basis of faecal egg counts, PCVs, mortality rates and eosinophil counts. In this experiment, however, it appeared that the Red Maasai lambs were getting less than their fair share of feed compared to the Dorper lambs and this could explain the 'tipping of the balance' which took place in favour of the Dorper. Although both the Dorper and the Red Maasai lambs used in this study were born at the NVRC, the latter were visibly smaller and since all the lambs were housed together they had to compete with the Dorpers for food. The clinical picture of dying lambs and the post-mortem findings of dead lambs pointed to inadequate nutrition combined with *Haemonchus* infection. This emphasises the importance of nutrition in comparative resistance studies and indicates a possible interaction between genetic resistance and nutrition. In this experiment, the eggs per adult female worm were quantitatively determined and the results showed that the superior resistance displayed by the Dorper was not directed towards worm fecundity. The resistance probably involved reduced establishment but since nutrition has been shown not to influence establishment (Abbott *et al.*, 1985a, b, 1986) the Dorpers were likely to have been expelling established worms early. In spite of the effects of nutrition that may have been present, the fact that Red Maasai lambs with over 1000 *H. contortus* worms did not survive in this experiment together with the fact that there were no

differences between the two breeds in the similarly designed fourth experiment, supports the contention that small, continuous infections stimulate better resistance in the Red Maasai than single large infections.

In the two comparative breed studies where three infections were given at intervals, two peaks of faecal egg counts occurred in response to the first infection but there was little apparent response to the third infection. The third infection was therefore a useful indicator of the development of resistance following the first two infections. In trickle infections, the response appeared to be more gradual before the highest peak was attained and this has been observed before (Dineen *et al.*, 1965).

No larvae were recovered from the abomasal mucosa in any of the studies reported in this thesis where worm counts were carried out. All immature forms found were in the ingesta and most of them had undergone sexual differentiation. Nevertheless studies at Naivasha, which is within a semi-arid climatic zone of Kenya, have shown the presence of arrested larvae in abomasal mucosa of sheep (Allonby and Urquhart, 1975; Gatongi, P., personal communication). It has been postulated however that after several years of laboratory passage *Haemonchus* larvae may lose the ability for arrested development (Benitez-Usher *et al.*, 1977). This was based on the fact that in cattle, the nematode *O. ostertagi* appears to lose the ability to inhibit at the early L4 stage after it has been cycled for several generations under laboratory conditions (Armour and Bruce, 1974). In our case this explanation may not be plausible since no arrested larvae were found even in grazing sheep.

Eosinophil responses were consistent in the studies in which these were assessed. Where the animals showed significant parasitological differences there were significant eosinophil differences as well. This suggests a role for this cell type in resistance. The association of eosinophil response and resistance to infection was seen many years ago. For instance, Hadwen (1925) found that these cells increased in circulation in colts infected with *'Ascaris' equorum* and

that the cells increased even more after a second dose of infective eggs. He suggested that the cells produced substances, some of which neutralised toxic parasite products while others immobilised or killed the worms. Indeed eosinophils produce various biochemical substances including enzymes (Wakelin, 1978; Tizard, 1987) and lgG and IgE bind to membrane receptors on the surface of these cells and thus stimulate them to release these substances (reviewed by Miller, 1990). Hadwen (1925) further observed that for tissue dwelling parasites the cells may be low or absent in the circulation, having been drawn into the invaded tissues in large numbers. In such cases the restoration of normal levels in circulation meant that the parasites had been overcome. In *H. contortus* infections an association of eosinophilia and resistance was suggested as early as 1942 by Andrews who found that recovery of sheep from experimental infections was accompanied by increased eosinophil numbers. In contrast, although infection stimulated an increased peripheral eosinophil response in random-bred and resistant lines of five to six month old sheep after primary and secondary *H. contortus* infections, the differences were not significant (Gill, 1991). In the same study, although differences in eosinophil numbers in the abomasal mucosa of the two lines of sheep were significant, there was no correlation between the number of cells and post-mortem worm burdens. Also, Adams (1993) found no difference in eosinophil levels in sheep between pre- and post-challenge *H. contortus* infection periods. Although Dineen *et al.* (1978) found a positive correlation between *T. colubriformis* worm burdens in three month old lambs and duodenal tissue eosinophilia, that is, a negative correlation with resistance, another study found no such relationship in either three or 10 month old lambs (Gregg *et al.*, 1978). Later work (Dawkins *et al.*, 1989; Buddle *et al*, 1992), however supported the view that eosinophilia is a sign of resistance in *T. colubriformis* infections. The present results showed that breed differences in eosinophil responses are not always clear-cut. In the trickle infection experiment where differences between the breeds were obvious, the

assays were carried out after cessation of the infections and the reason for such marked differences are not clear. The results further indicated that pre-infection eosinophil counts cannot be used as a marker of resistance since there was no relationship between pre-infection levels and response to subsequent infection in individual animals, a finding also reported by Buddle *et al.* (1992).

The 'self-cure' phenomenon in some trichostrongylid infections is a means by which infected sheep get rid of part or all of their existing worm burdens following acquisition of new larvae. In the present studies obvious 'self-cure' occurred at the same time in both Dorper and Red Maasai breeds, suggesting that the phenomenon is independent of breed differences in resistance to *Haemonchus*.

The results of regressing faecal egg counts on PCVs are not shown but in all of the studies the correlations were significant but moderate, varying between 0.30 and 0.65. The fact that variables are correlated does not necessarily mean that one causes the other; both could be related to some common underlying factor. The statistical significance observed in these experiments, despite the moderate correlation coefficients, could simply be due to the large number of observations involved (Fowler and Cohen, 1990). In haemonchosis, however, such significant correlations suggest a close relationship between the size of the worm burden and the degree of anaemia although variations in faecal egg counts do not provide unequivocal prediction of variation in haematocrits (Albers *et al.*, 1990). These workers found that at a certain stage of infection anaemia is associated not only with blood loss due to infection as measured by faecal egg counts but also with erythropoietic activity and the availability of iron. From their study, they further concluded that blood loss is the direct cause of poor productive performance in *H. contortus* infected lambs and therefore anaemia is the best predictor of the effect of *H. contortus* infection on productivity although other workers contend that the measurement of PCV may not always reflect true anaemia (Dargie and Allonby, 1975). In the study by Albers *et al.* (1990) there

was also evidence that the minimum PCV below which detrimental effects of *Haemonchus* infection are likely to be observed in Merino sheep is 28%. In our experiments, PCVs lower than these were commonly observed in housed animals, including uninfected sheep but grazing animals tended to have higher levels. It is likely that in our case sheep can have lower haematocrits than 28% and still do well as long as the diet is adequate in quantity and quality. Conway and Whitlock (1965) found that for sheep to survive *H. contortus* challenge, the minimum haematocrit values should be about 20%. They arrived at this figure on the basis of their observations that sheep can adjust to such an anaemia if not severely stressed and that resting sheep selectively sequester erythrocytes to maintain a PCV at this level. This may account for the relatively low PCVs in the sheep of the fourth experiment in which 20 Dorper and 20 Red Maasai lambs were infected with 5000 *H. contortus* three times at eight to nine week intervals, despite their otherwise reasonable performance.

The IgG responses, although not thought to be useful in the expression of resistance of sheep to gastrointestinal parasites, showed a noticeable pattern. Thus there were significant breed differences in the first experiment where there were large parasitological differences. Where parasitological differences were reduced as in experiment two or were absent as in experiment four, the breed differences in IgG responses were not consistently significant. It is only in the last experiment in which the Dorper was apparently more resistant than the Red Maasai, that the lgG responses were inconsistent with this pattern and the reason for this is not known.

The importance of diet, especially in housed sheep was evident in these studies. Where the animals were well fed differences between controls and infected groups in weight gains were not observed. Similarly some of the breed differences were not obvious. Also, where there was reason to believe that the Dorpers were better fed than the Red Maasai the Dorpers showed better resistance. In the latter experiment all of the animals were housed together so

that they would all be handled in the same way but the large number of lambs involved resulted in competition for food and probably significantly influenced the findings.

Overall, the Dorpers proved to do as well as the Red Maasai if adequately fed but in future experiments the issue of diet and possible competition between the breeds should be borne in mind. The results of the first experiment suggested that the Dorper has tended towards the Blackhead Somali in its resistance to *Haemonchus* and this has presumably reduced the differences in susceptibility between it and the Red Maasai.

Although individual variation within a breed was described only for the ewe experiment, significant individual variations were observed throughout the experiments for all the parameters examined, as evidenced in the tables containing the results of statistical analyses. These individual variations occurred even where there were no breed differences.

A simple method of quantitatively assessing the eggs per female was described and used in one of these studies. In this method intact adult females were homogenised in saturated salt solution using a glass tissue grinder and the eggs thus released quantified on a McMaster counting chamber. This method is likely to be easier and more reliable than the procedure of counting eggs in intact females under a microscope.

Finally, it seems relevant to examine the implications and difficulties associated with breeding for resistance. Genetic variability in the development of resistance to gastrointestinal parasites has been known for many years. However, it is only in recent years that detailed studies have been initiated to characterise the genetically- determined components of resistance using selective mating programmes in laboratory and domestic animals. For laboratory animals generation intervals are short and inbred strains are available but this is not the case with domestic animals. The long generation intervals mean that the progress of selection will be relatively slow. Thus Windon (1985) found that in sheep

undergoing selection for resistance against *T. colubriformis* the minimum generation interval is about two years, but this could be lengthened by adverse environmental conditions, such as drought. Added to this is the fact that genetic resistance of ruminants to gastrointestinal parasites is only moderately heritable and this is another limiting factor in the rate of selection progress.

Resources are also needed to maintain experimental animals and provide the labour required to test individual responsiveness. Use of small numbers of animals makes genetic analyses less accurate and increases the likelihood of inbreeding (Windon, 1985) even when this is not desirable. Carefully controlled laboratory conditions, although relatively easy to maintain when working with laboratory animals, are difficult to achieve with domestic animals. Despite these problems it is encouraging that a number of ovine selection programmes have been or are being conducted especially in Australia with *T. colubriformis* (Windon *et al.*, 1980; Windon, 1985) and *H. contortus* (Albers *et al.*, 1984; Woolaston *et al.*, 1990). The aim of the work by Albers *et al.* (1984; 1987) was to find out whether resistance was distinguishable from resilience, to what extent the two traits were heritable and the likely correlated effects of selective breeding for either of these traits. In general they found that resistance (determined by faecal egg counts and PCVs) and resilience (determined by liveweight gains) were distinct phenomena. However, they were related since selecting for resistance improved resilience. The heritability for resilience appeared to be too low to allow for substantial progress to be obtained for this trait by direct selection. Although there may be a decline in production during the induction of immunological responsiveness (on which resistance appears to be based), this is offset by the gains accrued when resistance mechanisms are finally established (Windon, 1985). Selection for resistance against *H. contortus* also appeared to increase resistance against *T. colubriformis* (Woolaston *et al.*, 1990) and possibly other nematodes but the converse is not necessarily true (Windon, 1985). With *T. colubriformis*, lines of sheep were established which

differed significantly in their level of resistance after vaccination at an early age with irradiated larvae. The high heritability estimates (0.4) obtained indicated that fairly rapid genetic gains were achievable in such selection programmes.

Metazoan parasites can survive inside a host long enough to mature and reproduce, a period usually longer than the time required for the host to mount an effective immune response. This led Dineen (1963) to suggest that in such cases the parasite displayed sufficiently reduced antigenic disparity between it and the host either by losing the relevant antigenic character or by acquiring the antigenic character of the host, or both, thus becoming less antigenically foreign to the host. It is speculated that this disparity is increased by selection in high responders resulting in reduced host tolerance to the parasite (Windon, 1985). This probably also accounts for the variability between and within breeds as observed in the present study and in other studies.

Since the host-parasite relationship is not static, the genetic diversity we intend to exploit in the host is equally likely to be present in the parasite (Albers *et al.*, 1984; Kassai and Sreter, 1992). Hence, just as the worms have evolved an ability to develop resistance against anthelmintics, they could theoretically also circumvent the genetic resistance mechanisms of the host. Such a situation would present difficulties particularly when the resistant animals have been produced by breeding as opposed to vaccination because response to a changing parasite would be slow. However, it is less likely that increased host resistance would exert selection pressure on the parasite population comparable in magnitude to the selection pressure exerted by highly effective anthelmintics. An integrated approach, encompassing existing methods and genetically resistant animals could prolong the effective life of each and probably reduce the overall costs (Albers *et al.*, 1984; Windon, 1985). It is also reasonable to assume that if host resistance depends on several genes and is expressed through a variety of mechanisms this resistance is less likely to break down. Also, perhaps as Windon (1985) observed, selection should not be specific so that it can protect against a

wide range of parasites, and therefore should be based in the field.

From the present studies, it appears that under natural conditions the Red Maasai is resistant to haemonchosis and breed substitution is therefore an immediate effective option for controlling ovine haemonchosis in endemic areas where sheep are kept for meat.

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APPENDICES

Appendix 1: Ingredients of tris-poisons antigen extraction buffer

---

2.0M Tris HCl pH 8.3 (Trizma hydrochloride, Sigma T6666)

2.0mM EDTA (Ethylene diamine tetra acetic acid, Sigma E9884

1.0mM PMSF in isopropanol* (Phenyl methyl sulphonyl flouride (Sigma P7626)

5.0uM Pepstatin A (Sigma P4265)

2.0mM 1,10 Phenanthroline (Sigma P9375)

5.0uM Leupeptin (Sigma L2884)

5.0uM Antipain (Sigma A6271)

25ug/ml TLCK (N -p-Tosyl-L-lysine chloromethyl ketone, (Sigma T7254)

50ug/ml TPCK (N-tosyl-L-phenylalanine chloromethyl ketone, (Sigma, T4376)

---

* Propan-2-ol (BDH 29694 6H)

Appendix 2: Ingredients of the carbonate bicarbonate coating buffer

---

A.Stock solutions

I. 0.2M Sodium bicarbonate 16.8g/l (BDH, 30151 5V)

II. 0.2M Sodium carbonate 21.2g/l (BDH, 30121 4L)

B.Working solution

17.0ml sodium bicarbonate + 8.0ml sodium carbonate made to 100ml with distilled water while adjusting pH to 9.6

Appendix 3: Ingredients of the phosphate-citrate substrate buffer

---

A. Chromogen solution

5.11g citric acid crystals (BDH 27780 4L)

9.15g di-sodium hydrogen orthophosphate (BDH 10249 4C)

in 1L distilled water

B. Substrate

OPD (o-Phenylene-diamine dihydrochloride, Sigma P8412)

C. Working solution

0.004g of OPD added to 10mls of chromogen solution and stored at -20^{0}C in the dark. Thawed and 5-10ul of 30% hydrogen peroxide added just before use

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Appendix 4: Individual total worm counts in animals of the four breed experiment which died during the period of natural exposure

Breed	Adult *H. contortus*			Immatures and *T. axei*	Total
	Female	Male	Total		
Romney Marsh	1515	2370	3885	590	4475
" "	240	180	420	70	490
" "	1640	1630	3270	90	3360
" "	2515	2495	5010	210	5220
" "	485	485	970	50	1020
" "	1350	1260	2610	760	3370
" "	Samples no available				
" "	1040	700	1740	270	2010
" "	670	620	1290	360	1650
" "	220	180	400	100	500
" "	740	400	1140	210	1350
" "	640	440	1080	30	1110
Dorper	3760	4710	8470	440	8910
"	460	440	900	50	950
Blackhead Somali	310	210	520	180	700
"	590	500	1090	200	1290
"	1070	1200	2270	180	2450
"	Samples not available				
"	290	230	520	150	670

Appendix 5: Occurrence of 'self-cure' in four breeds of sheep naturally exposed to *H. contortus* infection

Breed	Period	Fall in EPG		% Fall
		from	to	
Romney Marsh	Week 25-33	9241	1100	88.1
"	Week 41-45	14054	3711	73.6
"	Week 46-51	13600	980	92.8
Red Maasai	Week 7-10	1230	430	65.0
"	Week 12-13	1400	700	50.0
"	Week 17-19	1060	460	56.6
"	Week 38-39	860	110	*87.2
"	Week 42-45	770	110	*85.2
"	Week 46-48	1640	780	52.4
"	Week 51-52	2000	1200	40.0
Dorper	Week 11-14	4900	2100	57.1
"	Week 20-21	5300	2600	50.9
"	Week 24-26	5400	1400	74.1
"	Week 42-45	5000	540	*89.2
Blackhead Somali	Week 9-11	7200	3200	55.6
"	Week 12-15	6400	2300	64.1
"	Week 46-48	4400	1900	56.8
"	Week 42-45	2000	450	77.5

* 'Self-cure' according to the criterion of Preston and Allonby (1979a) of a 80% reduction in faecal egg counts within three weeks

Appendix 6: Analysis of variance of female and male worm lengths in Dorper and Red Maasai lambs of Group 1 and Group 2 in the experiment described in Chapter 7

Source	Group 1		Group 2	
	Probability			
	Males	Females	Males	Females
Breed	0.0111	0.0244	0.8810	0.5269
Sex	0.0647	0.1358	*	*
Animal(breed)	0.0200	0.0069	0.0005	0.0001

Model: worm length= breed sex animal(breed). Parentheses indicate animal nested within breed.

*female lambs from which worms were obtained were not enough for this analysis

Appendix 7: The least square mean worm lengths (± S.E.) in Group 1 and Group 2 lambs.

	Group 1		Group 2	
Breed	Males	Females	Males	Females
Dorper	13.0 (0.3)	22.9 (0.3)	16.2 (0.1)	24.4 (0.2)
Red Maasai	15.3 (0.5)	25.1 (0.7)	16.2 (0.1)	24.7 (0.2)