BREED RESISTANCE
TO
HAEMONCHIASIS //

BY
MARILYN FRANCES AUCUTT

A THESIS
SUBMITTED IN PART FULFILMENT
FOR THE DEGREE OF
MASTER OF SCIENCE
IN
THE UNIVERSITY OF NAIROBI
1979

DECLARATIONS

I, Marilyn Frances Aucutt hereby declare that
this thesis is my original work and has not been
presented for a degree in any other University.

Marilyn Aucutt

I, Dr John B Castelino hereby declare that
this thesis has been submitted for examination
with my approval as University supervisor.

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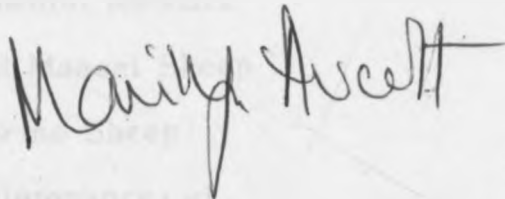
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JB Castelino

TABLE OF CONTENTS

SECTION ONE   39

The Influence of Breed on the Response of Sheep

to Experimental Infection with <u>Haemonchus contortus</u>

DISCUSSION   130

REFERENCES   139

ABSTRACT

Experiments were conducted to examine the nature of breed resistance to infection with Haemonchus contortus. Two breeds of sheep were chosen, Merinos - an exotic breed recently introduced into East Africa and Red Maasai - an indigenous breed. To avoid differences in resistance linked to haemoglobin type, all sheep were of haemoglobin type B.

In the first section two experiments are described, one using a 'Merino-adapted' strain of H. contortus and the other a 'Maasai-adapted' strain. No differences in response to the worm strain were noted in either breed so differences in response to infection between the breeds were assumed to be entirely due to the genetic make-up of the host. Response to infection was monitored using a combination of clinical, parasitological and radio-isotopic techniques. The sheep were all adults reared on contaminated pasture and thus previously exposed to H. contortus.

The Maasai breed was shown to harbour fewer Haemonchus worms than the Merinos and thus exhibited a milder disease syndrome than their Merino counterparts. Serial kills showed that initial establishment of worms was identical in both breeds but between 16 and 18 days after infection a period of worm expulsion occurred which resulted in a reduction in worm numbers to 4% of the infective dose in the Maasai but to only 37% in the Merinos. The resulting clinical syndrome was proportional to worm burden and there was no evidence that the

greater resistance of the Maasai was due to some superior compensatory mechanism.

The Merinos presented a typical picture of haemonchosis, namely hypercatabolism of albumin and a marked anaemia due to gastro-intestinal haemorrhage caused by the haemophagic activity of the worms. The anaemia was normocytic and normochromic later becoming macrocytic due to the release of large numbers of reticulocytes. The course and nature of the disease recorded was in agreement with that of other authors.

The difference in the pathogenesis of the disease between the two breeds of sheep was therefore due to the greater worm expulsion by the Red Maasai between 16 and 18 days after infection.

Further differences noticed between the breeds were assumed to be due to a greater degree of acquired immunity in the Maasai sheep; these differences included a later patency date of infection, considerable stunting of the worms recovered, and evidence of reduced worm fecundity.

In the second section the course of a primary infection was investigated by experimentally infecting yearling lambs raised worm-free with 250 L3/kg of H. contortus and challenging this infection 100 days later. The response of the lambs was measured as before but necropsies were not conducted.

A very marked difference in the pathogenesis of the two breeds was again demonstrated but the Maasai response was slower to develop than in the previous experiments. There was evidence that worm expulsion occurred over a period of several weeks in both breeds as judged by worm egg production (eggs per gram of faeces or epg) prior to challenge. The effect of challenge was to cause a marked reduction in epg 6 days later, representing a self-cure reaction of the previous infection, followed by an increase in epg as the challenge infection became patent.

In the Merinos considerable individual variation in response was recorded while the Maasai response was very uniform.

Evidence of the development of an acquired immunity was obtained from the gradual decline in epg prior to challenge and the later patency date of the challenge infection in the Maasai while patency of the primary infection occurred simultaneously in each breed. There was also indirect evidence of a reduced worm fecundity in the Maasai lambs after challenge.

The third section deals with a massive infection of H. contortus in Red Maasai and Merino sheep of 1,000 L3/kg. This was conducted to establish whether breed resistance in these breeds is dose-dependent as in haemoglobin-linked resistance to Haemonchus. Two experiments were carried out using a total of 10 Red Maasai and 11 Merino sheep and their response was monitored by clinical and parasitological means

including a necropsy of five individuals. The Maasai resistance remained unbroken while the Merinos included three fatal cases of haemonchosis and a generally much higher worm burden and more severe disease syndrome.

In the fourth section an experimental infection of H. contortus is described in two breeds of goats, the indigenous Small East African and an indigenous/exotic cross-breed, the Saanen/Galla. The Saanen/Galla were shown to harbour fewer worms and thus show fewer clinical symptoms of disease than the East African goats, but also maintained a higher epg. It was suggested that this was due to a difference in immune factors in the two breeds, some of which effect worm expulsion while others restrict worm fecundity.

In conclusion, the Red Maasai breed of sheep is much more resistant to infection with Haemonchus than the Merino breed. This resistance appears to be an immune response which develops rapidly and acts by a highly efficient mechanism of worm expulsion operating just before the infection becomes patent. A similar mechanism is also operative in the Merinos but is less efficient and the resulting disease syndrome is proportional to worm burden. In the Maasai acquired immune factors act upon the remaining worms causing stunting and reducing their reproductive potential. The Merino response is highly variable and some individuals may have a resistance at least temporarily comparable to that of the Maasai.

Acknowledgements

I am indebted to many people for their help and encouragement during the planning, execution and writing-up of this work. I am particularly grateful to my supervisor Dr John Castelino, senior lecturer at the University of Nairobi for his sound advice and continual encouragement. He gave freely of his time and knowledge and nothing was ever too much trouble. His encouragement was always timely and greatly appreciated.

I am also grateful to Drs Edward Allonby and John Preston at the Veterinary Laboratories for their advice, assistance and for providing the sheep, and to Jane and Edward Allonby for permission to quote some of their unpublished data. I would like to thank Professor W Mulligan and Dr George Urquhart of the Veterinary School, Glasgow University, for keeping me up to date on parallel work in Glasgow and for providing me with reprints I was unable to obtain in East Africa. I am also grateful to Dr Max Murray of ILRAD who gave me encouragement when it was most needed.

Much of the routine technical work was done not only efficiently, but cheerfully by Paul Amutulla and Geoffrey Muriithi - I am most grateful to them for their help, without which this work could not have been performed. They were assisted on several occasions by James and Ephantus Njoroge and John Kabanga. I am also grateful to John Njuguna and

INTRODUCTION

That some breeds of domestic animals and some individuals within these breeds thrive better than others in certain environments has undoubtedly been recognised since man first domesticated animals. Natural selection has favoured those individuals best adapted to their environment and man has furthered the process with selective breeding.

Factors influencing livestock survival can be grouped into broad categories related to climate, pasture and the availability of water but where such factors are not themselves limiting, disease becomes the overriding factor governing the development of animal husbandry.

The vast increase in human numbers during the past century combined with a higher expectation of living conditions has led to an ever-growing demand for protein. Since land suitable for agriculture is limited, emphasis is being increasingly put upon intensifying rather than expanding present resources and in particular upon reducing the enormous loss, both actual and insidious to disease.

Disease control is rarely simple and normally costly. In the case of many bacterial and viral diseases, effective vaccines have been manufactured and by careful vaccination programmes a specific disease may be eradicated entirely from large areas. In other diseases however, such as those caused by the

trypanosomes, the development of vaccines has been complicated by the rapid antigenic changes achieved by these parasites. In such cases control of the disease may depend on other approaches such as quarantine regulations with the prohibition of stock movements in and out of affected areas or on the eradication of a vector or reservoir host.

In the case of metazoan parasites and of helminths in particular, in spite of extensive and intensive research into the development of vaccines, progress has been slow and disappointing, due largely to the great numbers of antigens produced by these parasites and the very limited understanding of immunity to helminths in general. Effective irradiated vaccines have been available against the lungworms Dictyocaulus viviparus (Poynter, 1964) and D. filaria (Jovanovíc, 1964) since 1960 and more recently against the canine hookworm Ancylostoma caninum, (Miller, 1975) and the outlook is promising for vaccines against the hookworm Uncinaria stenocephala in dogs (Dow, Jarrett, Jennings, McIntyre and Mulligan, 1961) the gapeworm Syngamus trachea in poultry (Varga and Vetési, 1970; Feţeanu, Stoican, Negru and Verdeş, 1973) and the tapeworms Echinococcus granulosus and Taenia saginata (Cysticercus bovis)(Urquhart, 1964). However, mixed results have been obtained with vaccines against Haemonchus contortus (Urquhart, 1964; 1970; Urquhart, Jarrett and Mulligan, 1962) and Trichostrongylus colubriformis (Mulligan, Gordon, Stewart and Wagland, 1961) since immunisation is only possible in lambs over six months of age which have been raised worm-free

(Urquhart 1964).

In the case of the <u>Dictyocaulus</u> and <u>Ancylostoma</u> vaccines, which are the only ones actually in production at the present time, their usefulness is limited due to their short shelf-life, expense and problems of distribution. It will be some time before vaccines against helminths are readily available, particularly in developing countries where the need to protect stock against disease is most urgent.

Other approaches to disease control revolve around management procedures and chemical control measures, both of which, while feasible in developed countries, may be impractical in the Third World where expense and marginal pasture or climatic conditions combined with ignorance and small scale herding, militate against success.

In many parts of the world, the best hope of disease control lies in the stock animals themselves, by selecting for breeding those animals with the best natural resistance to disease. Regrettably, little thought has been given to this in the past and the practice of introducing stock from temperate zones into tropical areas may have aggravated the situation by introducing animals susceptible to local parasites and thus providing a population in which parasites can multiply.

In view of the obvious importance of natural resistance to disease, it is surprising that so little research has been done on

the subject. The reason for this is very probably the apparent solution to all problems of disease control offered by drugs and vaccines and it was not until their limitations were fully recognised that attention began to be focused on the utilisation of natural immunity.

The study described in this thesis involves the nematode Haemonchus contortus (Rudolfi, 1803), a trichostrongylid worm parasitic in the abomasum of ruminants, particularly sheep ,all over the world. It is a voracious blood sucker and occurs so widely and in such numbers that it has been described as the commonest and most pathogenic parasite of sheep (and probably goats) in the world (Kates, 1950; Whitlock, 1966) and is particularly important in the tropics (Dunn, 1969) where climate and traditional methods of animal husbandry favour its survival. In Kenya H. contortus occurs over all but the driest parts of the country and surveys indicate that the vast majority of sheep are infected (Allonby, personal communication; personal observation).

The adult worm is easily recognised in the abomasum, being the longest of the common stomach worms, the adult males measuring 12 - 15 mm and the females 16 - 18 mm. The males are a uniform red colour while the females resemble a barber's pole with the white ovaries spiralling around the blood filled gut. The mouth is simple and bears a dorsal lancet, cervical papillae are visible and the male bears a conspicuous bursa supported by rays.

The life cycle is simple and direct, eggs pass out of the host in the faeces, hatch in a few days under suitable conditions into the first stage larva (L1) which moults to become the second stage larva (L2) which moults again giving rise to the infective third stage larva (L3). The time taken for infective larvae to develop from the eggs is dependent on temperature and ranges from about 4 days at 37°C to 3 weeks at 11°C (Silverman and Campbell, 1959). Under humid conditions infective larvae may survive for many months on pasture while dry conditions cause high mortality (Soulsby, 1965).

The infective larvae are ingested by the host as it grazes and pass into the rumen where they exsheath. The parasitic L3 then pass into the abomasum where they begin to feed on the mucosa and grow rapidly, moulting twice more to become adult, about 18 days after ingestion (Soulsby, 1965).

Haemonchus contortus is a prolific egg layer, each female worm laying between 5,000 and 10,000 eggs per day (Martin and Ross, 1934; Lapage, 1968) while a single sheep may harbour up to 35,000 worms (Dargie and Allonby, 1975) each consuming an average of 0.05 ml of blood per day (Clark, Kiesel and Goby, 1962). The clinical symptoms produced by the worms are almost entirely due to blood loss (Fourie, 1931; Andrews, 1942) and the disease occurs either in an acute form which is usually rapidly fatal or in a more common chronic form leading to a general loss of weight and condition which may prove fatal on poor pasture (Allonby and Urquhart, 1975).

LITERATURE REVIEW

There are many reports in the literature of field
observations on the differences in worm burden, or apparent
worm burden, as judged by faecal worm egg output between
breeds of stock or between individuals of the same breed.
Many of these observations are rather cursory, such as that
of Stoll's (1929), in which he remarks in a paper on
Haemonchus contortus that "certain breeds, like the Dorset,
are held by some sheep men to be rather more resistant than
the average to 'worms'" and goes on to comment that sheep do
not appear to have been critically studied from a breed resistance
point of view - somewhat of an understatement perhaps,
particularly as critical studies were not conducted for another
thirty years after this paper was published. Following up Stoll's
work, Ross (1932) was moved to comment that natural resistance
(to H. contortus) is probably important; and Brandly and Waters
(1942) emphasised the importance of genetic make-up on disease
resistance and noted that this was "defined clearly in books
attributed to Hippocrates and for nearly 2,000 years found
repetition in medical literature" but then declined and has been
revived only in this century.

Many observations on breed or genetic resistance to
helminths have been based solely on worm egg production (eggs
per gram of faeces or epg) which is now known to be a rather
inaccurate measure of worm burden since one of the effects of
host resistance is to suppress worm fecundity (Dineen and

Wagland, 1966a). Similarly, venous haematocrit alone has been used as a measure of pathogenicity but Dargie and Allonby (1975) have shown that even during periods of moderate haemorrhage the haematocrit may remain steady producing a false impression of mild infection, while the disease itself is in fact severe and the haematocrit maintained by an increased erythropoietic response at the expense of the body's iron stores - a situation that rapidly leads to iron exhaustion and the development of a fatal anaemia.

One of the earlier references to breed resistance was made by Stewart, Miller and Douglas in 1937, in which they noted that Romney Marsh Sheep passed significantly fewer eggs of Ostertagia circumcincta than sheep of the Rambouillet, Southdown, Shropshire and Hampshire breeds while grazing together on the same infected pasture. They noted moreover that the variation within breeds was significant only in the susceptible breeds and postulated that a strain could be developed within a specific breed which is resistant to O. circumcincta while retaining the other features of that breed.

A more accurate measure of resistance to infection is provided by worm counts at necropsy. Several slaughter house surveys have provided information on worm load differences in various breeds. An example of this is the work of Patnaik, Mathur and Pachalag (1973) who noted that the Rambouillet breed of sheep in Rajasthan, India contained larger numbers of H. contortus and Oesophagostomum columbianum than the local

sheep or their cross-bred progeny.

Slaughter house surveys have the drawback that the history of the animals is unknown and factors such as exposure to infection, therapy, diet and weaning technique may all influence worm burden. More accurate assessments can be made from worm burdens of animals whose history is known and which have been reared under comparable conditions.

In France, Euzéby, Bussiéras, Moraillon and Boccard (1961) studied four breeds of sheep from April to September and noted that the Texel and Charmoise breeds harboured smaller numbers of H. contortus, Cooperia curticei and species of Trichostrongylus, Ostertagia and Nematodirus than the Southdown and Bleu du Maine breeds. In Nebraska USA however, Knight Vegors and Glimp (1973) found no significant difference in worm burdens of H. contortus between the Suffolk, Rambouillet, Targhee and Corriedale breeds grazing together during a two year period, but they noted that the local Navajo lambs had significantly fewer worms than the other breeds. In contrast, Scrivner (1964a) in Idaho, found that the Targhee (and Panama) breeds had fewer Ostertagia worms than the Suffolk, Hampshire and Rambouillet breeds grazing under similar conditions and that a lower worm burden was associated with lower epg counts.

A more detailed field study was conducted by Loggins, Swanson and Koger in 1965 in Florida - the first in a series of observations on natural and experimental infections of

H. contortus in different breeds of sheep, and one of the first really critical studies of breed resistance. Using data collected from a mixed flock of Southdown, Hampshire, Rambouillet and Florida Native sheep maintained at Gainsville from 1955, Loggins et al noted that the Florida Native sheep were more resistant than the other breeds as judged by worm burdens and epg and also by death loss and haematocrit. This is one of the first observations which refers to resistance to the effects of the parasites and not to parasite establishment alone.

These reports have established, therefore, that resistance to disease may be greater in some breeds and some individuals that others. However, in the case of breed differences, this presumes that the resistance is inherited - a generally accepted supposition, which is however supported by relatively little . experimental evidence.

Many authors have believed resistance to helminthiasis to be genetic including: Ross and Gordon (1933); Emik (1946; 1949); Shumard, Bolin and Eveleth (1957); Scrivner (1964a) and Radhakrishnan, Bradley and Loggins (1972) in sheep and Ross (1961) in cattle. Shumard et al further suggested that resistance was species specific, but Scrivner (1964b and 1967) found that resistance to Ostertagia was associated with resistance to H. contortus and perhaps Nematodirus as well. Emik (1949) using only weekly epg counts as a measure of resistance to trichostrongylids found that breed differences were significant and while heritabilities by the half-sib correlation method were

not significant he suggested that this did not preclude genetic control of resistance.

Emik (1949) also noted that male lambs were more infested than females but showed more significant differences between breeds, whereas Scrivner (1964b) noted that female lambs were more susceptible to Ostertagia than male lambs.

Whitlock in 1955 (a), was the first to establish the heritable nature of resistance to helminths. He called this the 'Violet factor' after a ram named 'Violet' whose progeny were more resistant to helminths than those of other rams. Whitlock used weight gain, venous haematocrit and epg as measures of resistance to a mixed field infection which was predominantly H. contortus.

Following up this work, Ross, Lee and Armour (1959) noticed that in Nigerian Zebu cattle, the offspring of one grandsire were more resistant to a naturally acquired helminth infection (which was predominantly H. contortus) than those of the other grandsires employed in breeding. This resistance was expressed as a lower epg count and higher weight gain and haematocrit. The resistance seemed to be specific and not related to a better overall performance by these calves.

Scrivner in 1964 (b) took this work a step further by selecting one resistant Targhee ram and one susceptible Suffolk ram, mating each of them with twenty-five range-type ewes and comparing the resistance of the resulting lambs on the basis of

epg and worm burden taken at intervals during experimental
infection with <u>Ostertagia</u> . He found that the initial worm
establishment of the Targhee-sired lambs was only two-fifths
of that of the Suffolk-sired lambs. Both groups underwent a
'self-cure' reaction to establish a threshold population of worms
which was very similar in each group, but established earlier
(week 12) in the Targhee-sired that the Suffolk-sired (week 25)
lambs; also the epg of the Targhee-sired lambs at its maximum
was only a third of that of the Suffolk-sired lambs. In 1967,
Scrivner repeated the experiment with 8 progeny of one Targhee
and 8 progeny of two Suffolk rams and was able to confirm his
previous (1964b) findings on inheritance of resistance through
the ram and also to link resistance to ostertagiasis with that to.
haemonchiasis.

In the work of Whitlock (1955a), Ross, Lee and Armour (1959),
and Scrivner (1964b and 1967) inheritance of resistance was
assumed to pass through the sire, however, there is no particular
reason to believe that helminth-resistance is sex-linked. Indeed,
Emik (1949) remarked that the dams rather than the rams were the
influential parents in transmitting resistance and Warwick, Berry,
Turk and Morgan (1949) recommended that intensive selection on
both sides of the pedigree was necessary since their work on
resistance to <u>H. contortus</u> over a period of nine years had
indicated the presence of polygenes of very low individual
importance in sheep while goats appeared to have a much higher
level of plus genes with possibly true dominance for resistance.
It is therefore interesting to note that in Scrivner's (1964b)

observations, one Targhee-sired lamb parallelled the Suffolks
in epg and worm burden and led Scrivner to comment that the
dam is also important in the inheritance of resistance.

Within breeds of sheep an interesting correlation between
haemoglobin type and resistance to haemonchiasis has emerged.
Sheep and goats show a clear polymorphism for haemoglobin
which is determined by two alleles, A and B combining to give
three phenotypes - AA, AB and BB (Evans, King, Cohen, Harris
and Warren, 1956; Allonby and Urquhart, 1976).

Haemoglobin A and B (HbA & HbB) have different oxygen
dissociation curves (Dawson and Evans, 1962) and HbA appears
to have a higher affinity for oxygen than HbB (Huisman, Vliet and
Sebens, 1958). Under severe anaemic stress HbA may be replaced
by another haemoglobin, HbC (Blunt and Evans, 1963; Braend,
Efremov and Helle, 1964; Vliet and Huisman, 1964) but the
precise advantage of this replacement is obscure since HbA and
HbC appear to have the same affinity for oxygen (Vliet and
Huisman, 1964).

The distribution of the haemoglobin alleles between breeds in
the same geographical region and even between groups of the same
breed in different geographical regions, shows a marked disparity
with the Hardy-Weinberg equation which is calculated on the
assumption that mutation and selection favouring one or the other
allele are absent. There is, for instance, a preponderance of
HbA sheep in some areas which are endemic for haemonchiasis

(Agar, Evans and Roberts, 1972; Allonby, 1974).

In a survey conducted on Merino, Dorper and Maasai sheep and East African and Galla goats in Kenya, Allonby (1974) noted that the majority of indigenous sheep and goats had a higher frequency of HbA than the exotic breeds which were predominantly HbB. However, the exotic Dorper sheep which have been particularly successful in East Africa were also found to have a high frequency of HbA alleles. This is in agreement with the findings of Evans and Blunt (1961) who reported that the gene frequencies in Southdown and Romney Marsh sheep imported into Australia from Britain were markedly different from those in the original British stock - with a higher frequency of HbA alleles in the Australian sheep. Apparently some form of selection is operative in these areas which favours HbA alleles and resistance to haemonchiasis would seem to be the obvious factor (Evans and Whitlock, 1964).

A number of experiments have been conducted to investigate the mechanism by which HbA sheep appear to enjoy an enhanced resistance to H. contortus and its effects when compared with sheep of the same age and breed of HbAB or HbB (Evans, Blunt and Southcott, 1963; Jilek and Bradley, 1969; Ross, 1970; Radhakrishnan et al, 1972; Bradley, Radhakrishnan, Patil-Kulkarni and Loggins, 1973; Altaif, 1975; Allonby and Urquhart, 1976; Altaif and Dargie, 1976; and Allonby, J., personal communication).

This resistance seems to operate at two levels - namely a reduced parasite load and secondly and of less importance, an enhanced resistance to the pathogenesis of infection. Altaif and Dargie (1976) were able to relate the pathophysiology of a primary infection observed in Scottish Blackface and Finn Dorset sheep of different haemoglobin types to worm burden, with the HbA sheep harbouring considerably fewer worms than the HbB sheep. Likewise, Altaif (1975) was able to correlate worm burden with pathogenesis in a study of a primary infection in Blackface lambs of different haemoglobin types and thus concluded that the resistance is innate rather than acquired and operates at the establishment level with resistance to the effects of the disease being of minor consideration. The lower establishment levels in HbA sheep are reflected by lower epg's as reported by Allonby (1974); Altaif (1975) and Altaif and Dargie (1976).

Evidence of immune phenomena have also been reported with reference to the haemoglobin type and include the occurrence of 'self-cure' which is more frequent and effective in the HbA types (Bradley et al, 1973; Allonby and Urquhart, 1976); a larger proportion of inhibited larvae in the worm population (Bradley et al, 1973); smaller adult worms (Radhakrishnan et al, 1972); delayed patency (Radhakrishnan et al, 1972); and an eosinophilic infiltration of the abomasum which is thought to be connected with 'self-cure' (Bradley et al, 1973).

Haemoglobin A sheep in general tend to be of a larger initial weight (Allonby, 1974; Altaif, 1975) and to be able to gain more

weight during infection (Bradley et al, 1973); have a higher initial haematocrit (Altaif, 1975; Altaif and Dargie, 1976) and/or be able to maintain a higher haematocrit during infection (Radhakrishnan et al, 1972; Bradley et al, 1973; Altaif and Dargie, 1976). They also appear to have a higher initial circulating red cell volume (Altaif, 1975), lose less whole blood to the worms (Altaif, 1975; Altaif and Dargie, 1976) and to suffer less hypercatabolism of albumin (Altaif and Dargie, 1976) than their HbAB and HbB counterparts.

The importance of haemoglobin type in resistance to helminthiasis should not be overlooked, but in all cases investigated the differences involved are at all times smaller than the differences between breeds for the same haemoglobin type (Bradley et al, 1973; Altaif, 1975; Altaif and Dargie, 1976). There is moreover, increasing evidence that resistance linked to haemoglobin type may only be operative at low infection levels, but be overwhelmed during high challenge (Altaif, 1975; Allonby, J , personal communication). In many cases however, the interpretation of data from investigations into breed resistance is complicated by the fact that the more resistant of two breeds contains more individuals of HbA and the relative importance of Hb-linked resistance versus breed resistance becomes confused (Jilek and Bradley, 1969; Ross, 1970; Altaif and Dargie, 1976).

AIMS OF THE STUDY

Previous studies on the resistance of various breeds of sheep to helminth parasites indicate that differences exist between breeds in the number of worms recovered and also in the pathogenesis of the resulting disease. However, it is unclear whether this resistance is largely related to the uneven distribution of HbA alleles amongst differenct breeds or to some other factor as yet undefined. If the latter is the case, how does this factor operate? Is it an innate or an acquired character of a physiological or immunological nature and at what level is it expressed, on worm establishment or the subsequent expulsion of the parasites or on a greater ability to compensate for their effects?

This study was therefore designed to try to answer some of these questions. The parasite chosen was Haemonchus contortus since it is the most common and pathogenic helminth parasite of sheep in the tropics (Kates,1950; Whitlock, 1966; Dunn, 1969) and because a breed of sheep indigenous to Kenya, the Red Maasai, had been observed under field conditions to be surprisingly resistant to H. contortus both in terms of clinical evidence of haemonchosis and in faecal egg output when compared with imported Merinos grazing the same pasture (Preston and Allonby, 1977).

The study was divided into several sections as follows:

First a general investigation was conducted into the pattern of the disease in the Red Maasai and Merino breeds of sheep using clinical, radioisotopic and parasitological parameters in adult sheep reared on contaminated pastures. Since the difference in response could have been related to the strain of parasite rather than the breed of host, two trials were run on similar sheep using first a 'Merino-adapted' and then a 'Maasai-adapted' strain of H. contortus.

Second the course of a primary infection was investigated by experimentally infecting and then challenging with H. contortus yearling lambs raised worm-free and measuring their response as before in order to compare a primary with a secondary infection.

In the third section, the response of sheep to a massive infection was investigated to determine whether breed resistance in these breeds is limited to low or moderate infection levels as in haemoglobin-linked resistance.

The fourth section involves an experimental infection of H. contortus in two breeds of goats.

MATERIALS AND METHODS

1 EXPERIMENTAL ANIMALS

In order to avoid possible differences in response to
infection linked to haemoglobin type as described by Evans,
Blunt and Southcott (1963) and Jilek and Bradley (1969), all sheep
were haemoglobin typed and only those of haemoglobin type B
were used.

i) Red Maasai Sheep

These are fat-rumped, reddish-brown hair sheep commonly
herded by the Maasai tribe but increasingly kept by other tribal
groups in Kenya and Northern Tanzania. Their size is
variable, ranging from 20 to over 50 kilograms in adult males.
Males are generally larger than females and wethers tend to be
larger than entire males. The build is long-legged with body
fat localised in the rump and to a lesser extent in the dewlap of
males.

The coat colour is predominantly reddish-brown and may be
a uniform colour or interspersed with white to a greater or
lesser extent. Pure white, black and white and pure black
individuals are not uncommon. The coat is coarse hair of a
variable length and is underlain by a white woolly undercoat
which is normally invisible in the dry season but may
proliferate during the cool, wet season to such an extent that

the hair coat is obscured. The coat on the face, belly and legs is sleek and short. The skin is tough and relatively thick.

Backward-curving horns are present in males and may reach impressive proportions; females occasionally have rudimentary horns. The ears are short and pendulous, tassels may be present and the tail is short and may be almost indistinguishable under the fat rump (Mason and Maule, 1960; personal observation).

Red Maasai sheep used in this study were purchased from local markets and farms. Their history was therefore not known but attempts were made to choose animals of the same weight, age and sex wherever possible.

ii) Merino Sheep

These are exotic wool sheep which are kept principally on the larger farms in areas of moderate rainfall but may also be seen grazing with indigenous sheep and goats in small flocks over parts of upland Kenya. The body weight varies from about 30 to 45 kilograms, the sexes are similar in size although males are usually larger than females. The build is shortlegged and stocky and fat is distributed throughout the body.

The coat is composed of white wool which may be 15 cm long and is fine and oily. On the face and hocks the coat is short and silky, but dense and woolly on the back and sides and thinner on

the belly. The skin below is delicate and pink.

Horns are present in males and some females and are backward-curving. The ears are small and pendulous. The tail is naturally long and slim but is normally docked off level with the rump.

All Merino sheep used in the study were obtained from the National Animal Husbandry Research Station, Naivasha. Their age and history were known and individuals of the same age, weight and sex were selected whenever possible.

iii) <u>Maintenance</u>

Sheep were routinely drenched with thiabendazole before being experimentally infected with <u>H</u>. <u>contortus</u> but examinations for blood parasites were not conducted.

Necropsy revealed only one individual containing some liver flukes and a tapeworm. Routine faecal examinations revealed the presence of coccidial oocysts in 0.3% of the samples.

All sheep were fed on Rhodes hay and lucerne supplemented with concentrates (Ewe and lamb nuts, Kenya Farmers' Association) <u>ad lib</u> and given continuous access to drinking water.

Stalls and cages were cleaned thoroughly every other day

to prevent reinfection with H. contortus larvae hatching from infected faeces.

iv) Weighing

Sheep were weighed once a week by suspension in a sling from a hanging balance (Salter, Model 235).

II PARASITOLOGICAL TECHNIQUES

i) Culture and Administration of Infective Larvae

In each experiment (with the exception of the 'Maasai-adapted' strain described in Section 1) larvae were obtained by culturing faeces taken from the Merino flock at the National Animal Husbandry Research Station, Naivasha. This strain of Haemonchus is known to have been endemic in this flock for ten years. Larvae cultured from this source were then passaged at least three times through a Merino wether which had been reared worm-free to one year of age at the Veterinary Research Laboratories, Kabete.

Infective larvae were cultured from faeces in jars at room temperature for two weeks. The jars were then filled with tap water at $30^{o}C$ and left to stand for two hours to allow the infective larvae to migrate out of the faecal pellets before being sieved off and concentrated by sedimentation. A clean culture could be made in this way if care was taken not to damage the

pellets. After concentration the number of larvae per ml of solution was assessed by counting the larvae from at least ten 0.1 ml aliquots taken after thorough mixing.

Infective larvae were administered per os by means of a 20 ml syringe attached to a length of rubber tubing. Care was taken to see that the larvae were swallowed and did not pass down into the lungs.

ii) Faecal Egg Counts

Estimation of worm eggs per gram of faeces (epg) was performed at Kabete by the/modified McMaster method. Three
following
grams of faeces were homogenised in 42 ml of tap water for two minutes, transferred to a stoppered jar containing glass beads, shaken vigorously and sieved to remove the coarse debris. The filtrate was then shaken and a 15 ml sample was centrifuged for 2 minutes at 350 g and the supernate poured off. The sediment was resuspended in an equal volume of saturated salt solution (NaCl) and several drops of amyl alcohol added to reduce air bubbles. Both chambers of a McMaster worm egg counting slide (Hawksley & Sons, London) were filled with the suspension using a Pasteur pipette. Care was taken to mix the suspension immediately before sampling and to hold the pipette horizontally while filling the chambers, since the salt solution causes the eggs to float. The total number of eggs in the two chambers was counted and multiplied by a factor of 50 to obtain the number of eggs per gram of faeces.

iii) <u>Necropsy</u>

Sheep were stunned with a captive bolt humane killer followed by severance of the jugular vein. The abdomen was opened and the abomasum removed by cutting through the duodenal and omasal junctions, taking care not to lose any of the abomasal contents. The abomasum was opened along the greater curvature and the contents were washed out with tap water into a bucket. The washings were made up to 2 litres and after thorough mixing two 200 ml aliquots were taken and formalised for later estimation at the Veterinary Research Laboratories, Kabete of the total worm burden.

The abomasal mucosa after washing, was scraped off and placed in a mixture of 3% HCl and 1% pepsin for 6 hours at 37°C to digest the mucosa and liberate any fourth-stage larvae. The digest was then diluted to 2 litres, mixed thoroughly and a 10% aliquot taken for counting. The total worm burden was assessed from this and a differential count made of fourth and fifth-stage larvae. The procedure is essentially the same as that described by Ritchie <u>et al</u> (1966). Between 30 and 40 worms from each animal were then measured by tracing from the screen of a projection microscope and correcting for the magnification.

III   HAEMATOLOGICAL TECHNIQUES

i) Packed Cell Volume (PCV)

Packed cell volume percentages were determined by the
microhaematocrit method. A capillary tube was three-quarters
filled with blood, heat-sealed at one end and centrifuged at
9,900 g for 5 minutes in a microhaematocrit centrifuge (Gelman
Instruments, Hawksley, England). After centrifugation the
tubes were read on a Hawksley Microhaematocrit Reader
(Hawksley & Sons Ltd, London) to determine the percentage PCV.

ii) Haemoglobin Typing

Haemoglobin typing was performed at Kabete by the
method described by Altaif (1975) on cellulose acetate strips
saturated with 0.15 M trisborate buffer (pH 9.0) in an
electrophoresis tank containing 0.05 M barbitone buffer (pH 8.5).
An equal volume of distilled water was added to each blood sample
to cause haemolysis and the samples were applied to the strips
near the cathode end with an applicator plate. A constant
voltage of 150 volts was applied for 40 minutes. The strips were
then removed, fixed for 5 minutes in a 5% aqueous solution of
trichloroacetic acid (TCA) and stained for a further 5 minutes
in a 3% aqueous solution of TCA containing 0.2% Ponceau S.
The strips were then washed three times in 5% aqueous acetic
acid and read against known standards.

iii) <u>Haemoglobin Concentration (Hb)</u>

Haemoglobin concentration in grams per 100 ml of blood
was estimated in a haemoglobinometer (Hemoglobinometer,
Coulter Electronics Inc, Hialeah, Florida) at Kabete.

iv) <u>Red Cell Count (RBC)</u>

Red cell counts in millions per cubic millimetre of blood
were estimated in an electronic particle counter (Coulter Counter,
Coulter Electronics Inc, Hialeah, Florida) at Kabete. The
blood was first diluted 1:50,000 with 'isoton' (Coulter).

v) <u>Mean Corpuscular Volume (MCV) and</u>
   <u>Haemoglobin Concentration (MCHC)</u>

These were calculated as follows:

$$MCV (\mu^3) \quad = \quad \frac{PCV \times 10}{RBC}$$

$$MCHC (\%) \quad = \quad \frac{Hb \times 100}{PCV}$$

IV   SAMPLING METHODS

i) <u>Blood and Plasma</u>

Samples were collected exactly 10 minutes after injection
of isotopes and at the same time each day thereafter. All samples

were counted in a gamma well-type scintillation counter (Packard) on the same day at the end of the experiment.

Approximately 5 ml of blood were withdrawn from the jugular vein into 10 ml evacuated glass tubes (Venoject, Jintan Terumo Co Ltd, Tokyo) containing heparin as anticoagulant at 20 International Units per ml and gently mixed by inversion. A carefully measured 1 ml sample was taken and diluted in 9 ml 0.05% sodium hydroxide in a labelled counting bottle. Great care was needed at this stage to ensure accuracy. The blood was drawn into a 1 ml pipette up to the 1 ml mark, the outside of the pipette carefully wiped with absorbent paper and the blood let down slowly into the sodium hydroxide so that it did not mix. When the pipette was empty some clean NaOH from the same bottle was drawn up to the mark and allowed to flow slowly out into the sample. This was repeated again, by which time the pipette was cleaned of blood. The sample was then mixed by inversion and the NaOH caused haemolysis and thus an even distribution of activity throughout the sample.

Plasma was obtained by centrifuging the rest of the sample for 10 minutes at 5,400 g (Select-a-Fuge 24, Bio-Dynamics Inc, Indianapolis, Indiana) and 1 ml of plasma was then transferred into 9 ml of NaOH as described for the blood sample.

ii) Urine

Urine was collected in buckets placed beneath the

metabolism cages which were emptied at the same time each day through a sieve into a measuring cylinder and rinsed with a little water which was then added to the rest of the urine and measured. The urine was mixed and a 10 ml sample taken by pipette and placed in a labelled counting bottle.

iii) Faeces

The sheep were fitted with harnesses supporting faecal collecting bags lined with polythene bags which were changed at the same time each day. The bags were weighed along with any loose pellets obviously belonging to the animal but these were not added to the rest of the faeces. The faeces were mixed thoroughly and a 10 gram sample taken and compressed into a counting bottle.

V   RADIOISOTOPIC TECHNIQUES

i)   Measurement of Red Cell Turnover

The use of ^{51}Cr labelled red cells to measure red cell survival both in man and domestic animals has been widely used and is now a well accepted and established technique (Jennings, 1968; IAEA, 1972). The ideal tracer for such studies should attach firmly to the red cells, not be reabsorbed or reincorporated into other red cells, not be released until the cells are destroyed or excreted and most important, not damage the cells. No label has been found which fully meets these conditions but ^{51}Cr is the most suitable to date and although not providing

strictly quantitative data, is particularly useful for comparative studies especially where gastro-intestinal haemorrhage is involved (Jennings, 1968). Labelling of red cells with ^{51}Cr occurs when anionic hexavalent chromium penetrates into the cells and is reduced to cationic trivalent chromium which becomes bound to the globin fraction of haemoglobin (Gray and Sterling, 1950).

Labelling of red cells with ^{51}Cr has the disadvantage that although the label attaches firmly to the cells in vitro and is not reutilised after destruction of the erythrocytes, it is gradually eluted from the red cells after labelling (IAEA, 1972). This elution takes place in two phases, a rapid initial phase lasting 1 - 3 days and a more gradual exponential loss (Tucker, 1963; Jennings, 1968). Label eluted from red cells in the circulation is rapidly excreted in the urine (Jennings, 1968). In comparative studies, providing the initial rapid elution phase is ignored, no correction need be made for the exponential phase of elution (Jennings, 1968).

Estimation of circulating red cell volumes, apparent red cell half-life and red cell loss due to gastro-intestinal haemorrhage can all be calculated by using ^{51}Cr labelled red cells injected into an animal.

Circulating blood and red cell volumes are calculated by the dilution principle based on the comparison of total injected activity with that of a blood sample taken after the labelled cells

have undergone thorough mixing in the animal's circulation.
This method gives an accurate estimation of circulating red cell
volume but tends marginally to underestimate the blood volume
because the venous haematocrit is actually an overestimate of
the body's true circulating red cell : plasma ratio since PCV in
the capillary beds is slightly lower than that in the veins, but
for comparative studies blood volume measured in this way is
quite adequate (Altaif, 1975). In order to correct for weight
differences between individuals in a study, circulating red cell
volumes are usually expressed on a body weight basis.

Apparent red cell half-life is estimated by calculating red
cell activity per day post-injection as a percentage of the
equilibrium value obtained after mixing of the labelled cells in
the circulation. A semi-logarithmic plot is then made against
time and the apparent half-life estimated from the best straight
line formed after the initial rapid elution phase. This is
illustrated in Figure 1. The apparent half-life is the time taken
for the activity of the red cells to decrease by 50% and is
'apparent' rather than 'true' due to the elution of isotope from
the red cells and is therefore an under-estimate of the true
value (Tucker, 1963). On the assumption that elution in
animals of the same species treated in the same way is
comparable, this nevertheless provides a very useful index of
red cell survival.

Since any ^{51}Cr from the red cells released by elution or
destruction of the cells in the circulation is excreted in the urine,

^{51}Cr appearing in the faeces provides a quantitative measure of gastro-intestinal haemorrhage. In sheep there is evidence that some isotope (c 12%) may actually be resorbed from the gut leading to an underestimate of haemorrhage, but this seems to be balanced by a similar leakage of isotope into the gut which is not related to haemorrhage (Clark, Kiesel and Goby, 1962). From the blood activity and PCV, faecal 'clearances' of blood and red cells can be estimated by calculating the quantity of blood necessary to provide the activity of ^{51}Cr in the faeces. Assuming that blood lost into the gut takes about 24 hours to appear in the faeces, faecal blood clearance (in ml) is calculated by dividing faecal activity by the previous day's blood activity per ml.

ii) Labelling of Erythrocytes with ^{51}Cr

Sheep were prepared by shaving both sides of the neck around the jugular veins. Approximately 30 ml of blood were withdrawn from each sheep into heparinised evacuated tubes, centrifuged for 5 minutes at 1,400 g and the plasma and white cells removed with a Pasteur pipette and saved. The red cells were washed three times in 0.85% sodium chloride to remove all traces of plasma, resuspended in physiological saline and incubated with 1 ml of ^{51}Cr as sodium chromate ($Na_2^{51}CrO_4$, Radiochemical Centre, Amersham, England) representing 100 - 200 μCi per ml of packed cells in a water bath for 30 minutes at 37°C. The solution was mixed gently every ten minutes and after labelling the red cells were washed three times to remove all unbound isotope and reconstituted with their own plasma. The

labelled cells were injected back into their respective donors
via a jugular catheter (Gauge 14, Portex Ltd, Hythe, England)
which was flushed through with saline before being withdrawn.
Exactly 10 minutes after injection a blood sample was taken from
the opposite jugular vein to serve as the equilibrium value used
in calculating red cell volume and half-life. Before injecting
the labelled red cells into the sheep a 1 ml sample (weighed to
the nearest milligram) was diluted to 200 ml in a volumetric flask
with 0.05% NaOH and the weight of red cells injected into the
sheep was taken for estimation of blood volume.

iii) Measurement of Plasma Loss

One of the marked effects of gastro-intestinal parasites is
to cause hypo-proteinaemia due to plasma leakage into the gut
(Mulligan et al, 1967; Nielsen, 1968). The method usually
applied to measure this leakage is to label the host's plasma with
a radioactive isotope whose subsequent appearance in the gut
can be measured and used to estimate plasma loss (Nielsen, 1968).
The most useful isotope for this is radioiodine which, providing
the thyroid gland has been previously blocked with stable iodine,
is not reutilised once released by protein degradation and is
excreted in the urine (McFarlane, 1965; Freeman, 1967; IAEA,
1972).

The presence of radioiodine in the faeces is thus an indica-
tion of plasma loss into the gut and can be measured quantitatively
in the same way as red cell loss by dividing the faecal activity of

radioiodine by the previous day's plasma activity.

In practice, labelled albumin rather than plasma is injected into the host. One method of preparing this is by treating the albumin under slightly alkaline conditions with iodine monochloride to which has been added the radioactive iodine as carrier-free iodide. The radioiodine is then substituted into the tyrosine residues of the albumin and the label becomes firmly attached during the life of the protein (McFarlane, 1958).

iv) Labelling of Albumin with ^{125}I

Commercial sheep albumin was trace-labelled with radioiodine by the iodine monochloride method described by McFarlane (1958).

Materials:

1. Carrier-free radioiodide obtained from the Radiochemical Centre, Amersham, England as thiosulphate-free Na^{125}I.

2. Iodine monochloride as a solution containing 0.42 mg I/ml as ICl in molar NaCl and approximately 0.01 N with respect to HCl.

3. Glycine buffers -

    A: (pH 8.5) 9 ml molar glycine in 0.25 M NaCl + 1 ml N NaOH.

    B: (pH 9.0) 8 ml molar glycine in 0.25 M NaCl + 2 ml N NaOH.

4. A 2% solution of sheep albumin (Cohn Fraction V, Koch-Light Laboratories Ltd, Colnbrook, England) prepared by dissolving 600 mg freeze-dried commercial sheep albumin in 30 ml isotonic saline buffered with 15 ml glycine buffer B.

5. Bovine albumin (Cohn Fraction V pure, Koch-Light Laboratories Ltd. Colnbrook, England).

Method:

Ten mCi of radioiodide was added to 2.5 ml of freshly-prepared ICl to which 10 ml of glycine buffer A was then added and the mixture was immediately mixed with the sheep albumin solution by jetting the iodinating solution into the albumin with a Pasteur pipette. This preparation was then transferred into dialysis tubing to which had been added 2 grams of 'carrier' protein (freeze-dried bovine serum albumin) to reduce the specific gravity of the labelled albumin to less than $5\mu Ci/mg$ and thus reduce the possibility of radiation decomposition (Freeman, 1967). This was then dialysed for 48 hours at $4^{o}C$ against two 20 litre changes of isotonic saline to remove unbound iodide and centrifuged for 30 minutes at 350 g before injecting approximately $200\mu Ci$ ^{125}I into each sheep via a jugular catheter.

VI CALCULATIONS

i) Red Cell Loss per Day

Tables were constructed for each animal as follows, where 'activity' refers to ^{51}Cr counts per second.

	A	B	C	D	E	F	G	H
D A Y	Faecal Act/gm	Total wt of faeces	Total faecal Act	Blood Act/ml	PCV	RBC Act/ml	RBC Clearance	RBC Clearance Means
0								
1								
2								

Day 0 was the day on which isotopes were injected into the sheep. Calculations were performed as follows, where letters refer to the column and numbers to the day.

A_1 x B_1 = C_1 = Total faecal activity for days 0 - 1

D_0 x $100/E_0$ = F_0 = RBC activity on day 0

C_1/F_0 = G_0 = ml red cells lost to the gut on day 0

Column H: means were calculated to eliminate wide fluctuations from day to day as follows:

$$\frac{G_1 + G_2 + G_3}{3} = H_2 \quad \text{and} \quad \frac{G_2 + G_3 + G_4}{3} = H_3$$

ii) <u>Apparent Red Cell Half-Life</u>

The red cell activity per day was calculated as a
percentage of the 10 minute equilibrium sample. The apparent
red cell half-life ($t\frac{1}{2}$) was calculated from the regression
coefficient (b) calculated from a plot of the logged percentages
against time in hours as:

$$t\frac{1}{2} = \frac{\log 2}{b}$$

iii) <u>Plasma Loss per Day</u>

This was calculated in essentially the same way as red cell
loss by dividing total faecal ^{125}I activity by plasma ^{125}I activity
of the previous day to obtain plasma loss in ml during that 24
hour period.

iv) <u>Apparent Plasma Half-Life</u>

This was calculated in the same way as red cell half-life,
by plotting daily ^{125}I plasma activity as a percentage of the 10
minute sample against time.

v) <u>Circulating Blood and Red Cell Volumes</u>

The weight of labelled blood injected into the sheep was
obtained by weighing (to the nearest milligram) the syringe
containing the blood before and after injection, making sure that
no blood was lost in the process and that loss by evaporation

was minimal.

Comparison of the 10 minute sample with a standard dilution gave the dilution of ^{51}Cr in the sheep and thus its blood volume. The standard dilution was prepared by injecting a weighed 1ml sample of the labelled blood into a volumetric flask and filling this exactly to the mark with 0.05% NaOH. This was then mixed and a 1 ml sample pipetted into 9ml NaOH as already described.

Calculation:

$$\text{Total Standard Activity} = \text{Activity of sample} \times \frac{\text{Dilution of sample}}{\text{Size of sample}}$$

$$\text{Eg} = 1,000 \text{ counts/sec} \times \frac{200 \text{ ml}}{1 \text{ ml}} = 200,000 \text{ counts/sec}$$

$$\text{Total Injected Activity} = \text{Activity of standard} \times \frac{\text{wt injected}}{\text{wt of standard}}$$

$$\text{Blood Volume} = \frac{\text{Total Injected Activity (counts/sec/ml)}}{\text{Activity of 10 minute sample (counts/sec/ml)}}$$

$$\text{Red Cell Volume} = \text{Blood Volume} \times \frac{\text{PCV}}{100}$$

VII   STATISTICAL METHODS

Significance tests were performed by the 'Students' t-test method (Bailey, 1959) taking $P < 0.05$ as the lowest acceptable level of significance and $P < 0.001$ as being highly significant. The symbols ø indicate that the test was significant ($P < 0.05$),

⊕⊕ highly significant (P<0.001), ⊕⊕⊕ very highly significant (P≪0.001) and N/S not significant. Means are quoted ± their standard errors where appropriate.

Significance tests of time-dependent variables such as red cell loss or PCV over a period of time were calculated by performing a t-test on the mean value for each individual animal over the period in question. Standard errors of percentages were calculated using the method described by Guilford (1965).
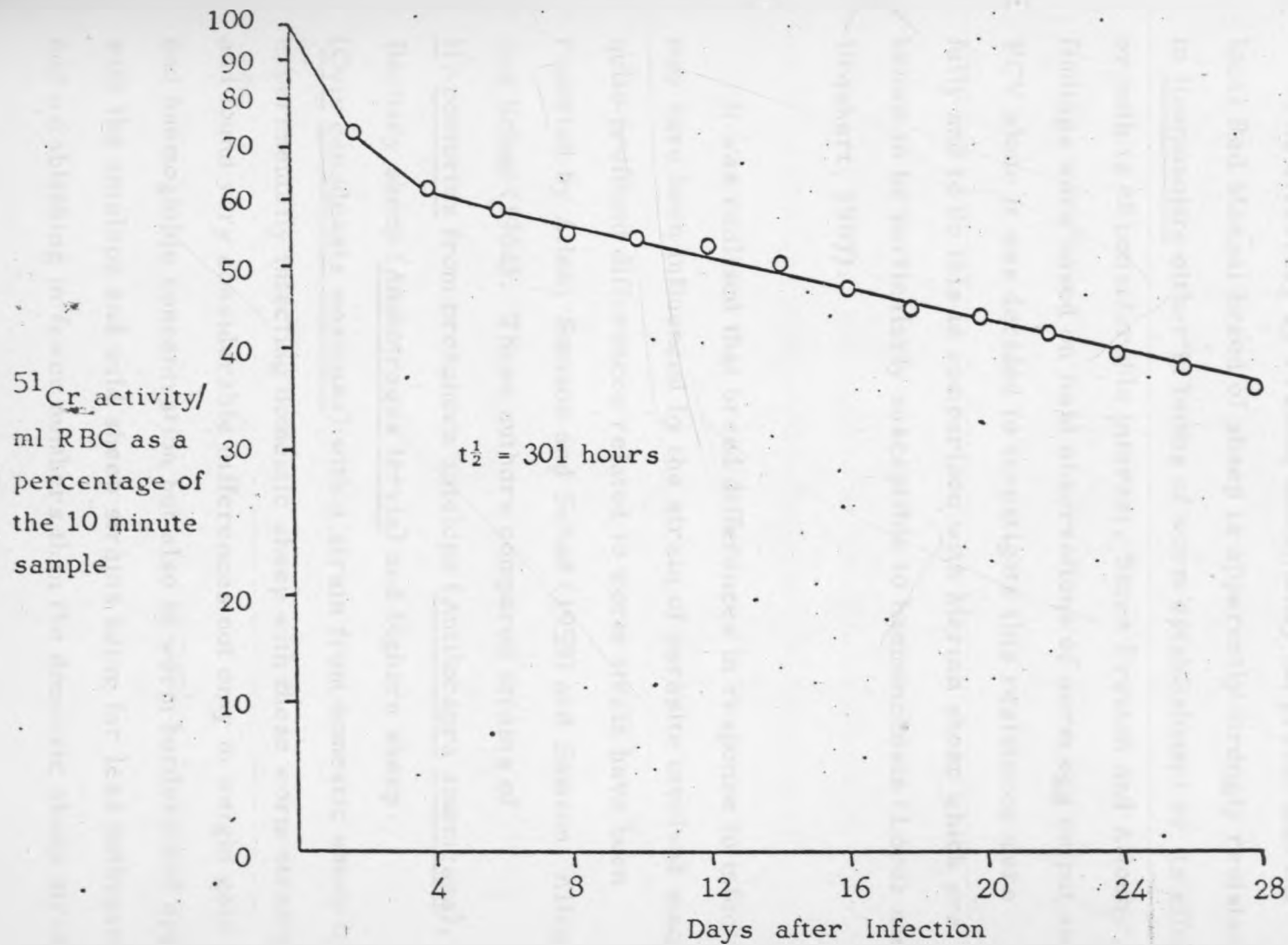
Figure 1: Disappearance of ^{51}Cr labelled red cells from the circulation of a normal sheep (adapted from Altaif 1975)

. THE INFLUENCE OF BREED ON THE RESPONSE OF SHEEP TO
EXPERIMENTAL INFECTION WITH HAEMONCHUS CONTORTUS

The discovery by Preston and Allonby (in press) that the
local Red Maasai breed of sheep is apparently strongly resistant
to Haemonchus either in terms of worm establishment or its effects
or both is of considerable interest. Since Preston and Allonby's
findings were based on field observations of worm egg output and
PCV alone it was decided to investigate this resistance more
fully and to do this in comparison with Merino sheep which are
known to be particularly susceptible to haemonchosis (Lopez and
Urquhart, 1967).

It was realised that breed differences in response to infection
may have been influenced by the strain of parasite involved since
quite profound differences related to worm strain have been
reported by Allen, Samson and Schad (1959) and Samson, Allen
and Schad (1964). These authors compared strains of
H. contortus from pronghorn antelope (Antilocapra americana),
Barbary sheep (Ammotragus lervia) and bighorn sheep
(Ovis canadensis mexicana) with a strain from domestic sheep by
experimentally infecting domestic sheep with these worm strains,
and found very considerable differences not only in weight gain
and haemoglobin concentration but also in worm burdens and epg
with the antelope and wild sheep strains being far less pathogenic
and establishing in fewer numbers than the domestic sheep strain.

Indeed, Allen and Samson (1961) and Allen, Samson and Wilson (1970) were able to induce a significant immunity to H. contortus by experimentally infecting domestic sheep with these less pathogenic strains. It was therefore possible that a similar situation might exist between worm strains adapted to different breeds of sheep.

Furthermore, the difference in the length of time in which the indigenous sheep and the recently imported Merino sheep had been associated with the local strain of H. contortus could be extremely important. Whitlock (1949;1955b) suggested that host and parasite evolve to a state of balance characterised by an asymptomatic carrier state which he called parasitiasis, while parasitosis (the disease condition) is evidence of conflict. It was therefore possible that the observed difference in response was due to the shorter time in which Merino sheep and the Kenyan strain of H. contortus had been in contact. For this reason a strain of H. contortus was cultured from faeces of Maasai sheep in Narok District where Merino sheep do not occur and the course of infection monitored in Maasai and Merino sheep while all other trials were conducted using a strain known to have been endemic in Merino sheep at the National Animal Husbandry Research Station, Naivasha for ten years.

METHODS AND MATERIALS

i)   Experiment 1

This experiment consisted of two parts, firstly an
investigation of the course of an infection with H. contortus
conducted on four Red Maasai and four Merino sheep and
secondly, serial necropsies conducted on sixteen sheep of each
breed to examine worm numbers at different stages in the course
of infection.

The sheep used were all wethers of haemoglobin type B,
aged between two and four years at the start of the experiment
whose weights ranged from 11.0 to 31.0 kg (mean weights:
Maasai 19.4 ± 1.39 kg; Merinos 24.2 ± 1.45 kg) which had
been reared under normal management practice. The Merinos
had been grazed on pasture at Naivasha known to be contaminated
with Haemonchus contortus and the Red Maasai sheep were
purchased from Ngong where H. contortus is also known to
occur. The history of the Maasai sheep was not known but it
was assumed that they had been exposed to infection since they
had been reared in the vicinity of Ngong.

The sheep were brought indoors and drenched with
thiabendazole ('Thibenzole' - Merck, Sharp & Dohme,
Australia, Pty Ltd) at 100 mg/kg to eliminate any existing
worms three months before the start of the experiment. They
were maintained throughout the experiment on Rhodes hay and

lucerne _ad lib_ supplemented with concentrates (Ewe & Lamb
Nuts, Kenya Farmers' Association) and given free access to
drinking water.

In both parts of this experiment, a 'Merino-adapted' strain
of H. contortus was used which is known to have been endemic
in the Merino flock at the NAHRS, Naivasha for the past ten
years. This was collected and passaged through a Merino
wether which had been reared worm-free at the Veterinary
Research Laboratories, Kabete.

Infective larvae were administered at a dose rate of 350
infective larvae per kilogram body weight (350 L3/kg), an
infection rate which has been found to be comparable to natural
infection levels (Allonby, personal communication). The practice
of administering larvae on a body weight basis is now widely
accepted and based on a general assumption that heavier animals
tend to consume more food and thus under field conditions, more
larvae, and eliminates any advantages enjoyed by larger animals in
compensating for the effects of the parasites (Altaif, 1975;
Altaif and Dargie, 1976).

Part One:

The course and pathogenicity of an experimental infection
of 'Merino-adapted' Haemonchus contortus was monitored in
four Red Maasai and four Merino sheep using ^{51}Cr labelled
erythrocytes and ^{125}I labelled plasma, the appearance of which

in the faeces was used to measure gastro-intestinal
haemorrhage.

The sheep were confined in metabolism cages and fitted
with faecal collecting bags two weeks before isotopic labelling
to allow them to adjust to the cages. One week before the
injection of isotopes until the end of sampling the sheep were
given drinking water to which had been added a dilute salt
solution to stimulate drinking and a 0.05% solution of potassium
iodide to block absorption and storage of radioiodine by the
thyroid.

Autologous ^{51}Cr labelled red cells and ^{125}I labelled
albumin were injected simultaneously into the jugular vein of
each sheep and the sheep were infected with 350 L3/kg of
H. contortus five days later. Each day for 43 days after
labelling haematocrit was measured and a blood, plasma, urine
and faecal sample taken and total daily urine and faeces output
measured. Sheep were weighed and a blood analysis performed
once a week and twice a week faecal output of worm eggs per
gram (epg) was monitored. All samples were counted in a
gamma well-type scintillation counter (Packard) on the same
day at the end of the experiment and calculations made to
assess blood and plasma loss. The sheep were drenched with
thiabendazole at the end of the experiment.

Part Two:

Worm burdens during the course of an experimental
infection with 'Merino-adapted' H. contortus were assessed
by conducting serial necropsies of two Red Maasai and two
Merino sheep of comparable weights at intervals between 12 and
28 days after infection. The sheep used in the pathogenicity
experiment were included in this experiment. Infection with
350 L3/kg took place four and a half weeks after drenching at
the end of the pathogenicity experiment.

ii)   Experiment 2

Four Red Maasai and four Merino sheep were infected with
a 'Red Maasai-adapted' strain of H. contortus at an infection
rate of 350 L3/kg and their response was monitored at the
Veterinary Research Laboratories, Kabete by epg alone.

The sheep were all wethers of haemoglobin type B aged two
years at the start of the experiment which had been reared on
pastures known to be infected with H. contortus. The Merinos
were obtained from NAHRS, Naivasha and the Red Maasai from
Narok. The sheep were drenched with thiabendazole and kept
indoors for six months before infection.

The 'Red Maasai-adapted' strain of H. contortus was
cultured from faeces of infected Red Maasai sheep in Narok
District. Since this area is too arid for Merino sheep and

stock movement is limited, this strain was presumed not to have been in contact with sheep other than Red Maasai.

RESULTS

i) Experiment 1

The fluctuations in mean faecal egg counts (epg) of the four
Red Maasai and four Merinos used in the pathogenicity study are
shown in Figure 2. The pattern in the two breeds is similar but
of a very different magnitude, the Maasai epg running close to
4% of that of the Merinos and never rising above 12% of the
Merino value. In the Merinos a peak of 3,400 epg occurred on
day 35 after which there was a brief decline followed by a sharp
rise to 8,000 epg. The Maasai showed a similar steady rise to a
maximum on day 35 but this was only 700 epg and was succeeded
by a fall to 100 epg.

In the Merinos 3 out of 4 sheep had positive egg counts on
day 21 while eggs were not observed in the faeces of any Maasai
sheep until day 28 and it was not until day 35 that all four had
detectable worm eggs.

The mean total faecal egg output showed a similar trend and
is illustrated in Figure 3 in relation to mean body weights, PCV
and daily loss of red cells. The changes in body weight
between the two breeds were not significantly different, due
probably to the ad lib feeding regime and short duration of
infection.

The mean PCV declined in both breeds during the first 21

days post-infection although this was slightly more marked in
the Merinos (ie a fall from 32% to 13% in the Merinos compared
with a fall from 31% to 17% in the Red Maasai). Thereafter
there was a gradual increase of about 3% in both breeds by day
38. There was no significant difference in the mean PCV of
the Maasai and Merinos before day 16 (Maasai 26.3 ± 1.69%;
Merinos 25.5 ± 1.83%) but a significant difference (P<0.05) was
noted after day 16 (Maasai 18.9 ± 1.39%; Merinos 13.9 ± 1.05%).
The mean fall in PCV was significant in both breeds (P<0.002).

A more detailed picture of the anaemia is shown in
Figure 4 where PCV can be seen in relation to haemoglobin
concentration (Hb), red cell count (RBC), mean corpuscular
volume (MCV) and mean corpuscular haemoglobin concentration
(MCHC). (A delay in processing samples taken on day 21 led
to their exclusion from the graph.) The nature of the anaemia is
typical of haemonchosis, the decline in PCV being paralleled by
a similar fall in Hb and RBC in both breeds. The anaemia is
essentially normocytic and normochromic, but a trend towards
a macrocytic hypochromic anaemia in the Merinos is obvious
after the infection became patent.

The mean daily loss of red cells (Figure 3) rose in both
breeds from day 10 and increased progressively and at the
same rate in each breed to a daily loss of approximately 7 ml
by day 15. With a mean PCV of 20% at this time this
represents a loss of about 35 ml of whole blood per day.
From day 16 onwards the pattern of red cell loss was

different in each breed. In the Merinos the red cell loss continued to rise and apart from a brief decline around day 20, increased steadily to a maximum of 21 ml on day 30, dropping abruptly on day 31 to 16 ml, and remaining near this level until the end of sampling on day 35. In the Red Maasai the mean daily red cell loss followed a very similar pattern to that of the Merinos but at a lower level after day 16, reaching a maximum of 12 ml on day 32 and falling to 8 ml by day 35. The difference between mean red cell loss before (Maasai 2.7 ± 0.70 ml; Merinos 3.0 ± 0.31 ml) and after (Maasai 7.6 ± 2.20 ml; Merinos 12.8 ± 2.48 ml) day 16 was significant only in the Merinos ($P < 0.01$), and the small sample size precluded the demonstration of any statistical significance between the breeds for either period.

The effect of this increasing loss of red cells is further reflected in the red cell half-life ($t\frac{1}{2}$) illustrated in Table 1. The pattern of red cell loss and therefore $t\frac{1}{2}$ was found to fall naturally into three sections corresponding to the prepatent period with minimal blood loss up to day 13, the early fifth-stage larval and early patent period up to day 27 and the fully patent, chronic stage after day 27. The $t\frac{1}{2}$ was calculated for each of these stages separately as well as for the whole period of infection. Except for the first period, the Maasai $t\frac{1}{2}$ was longer than that of the Merinos but differences were not statistically significant.

Plasma loss, illustrated in Figure 5 shows a clear and

significant difference between breeds after day 16 (P <0.05)
while the difference is not significant before this time, and the
difference before and after day 16 is only significant in the
Red Maasai (P <0.01). In the Maasai the mean plasma loss
after day 16 was 14.6 ± 2.80 ml while that of the Merinos
fluctuated around a mean of 45.0 ± 13.87 ml. The very high
plasma losses between days 5 and 15 are particularly
interesting and presumably reflect the feeding activities of the
fourth-stage larvae causing very considerable plasma leakage
into the gastro-intestinal tract (up to 77 ml per day in the
Merinos) but very little loss of erythrocytes (1.9 ml in the
Merinos on the same day).

The plasma $t\frac{1}{2}$ is illustrated in Table 2. As with the red
cell $t\frac{1}{2}$, differences were not statistically significant but the mean
values indicate a breed difference which increases during the
course of infection.

Total worm burdens recovered from two sheep of each breed
necropsied at intervals from 12 to 28 days after infection are
shown in Figure 6 as a percentage of infective larvae
administered. Maximum worm recovery occurred on day 12 (mean
of $5,400 \pm 3,000$ worms or $61.7 \pm 34.37\%$ of the infective dose in
the Red Maasai and $6,800 \pm 400$ worms or $76.2 \pm 30.12\%$ of the
infective dose in the Merinos). After this worm recovery
declined in both breeds reaching an equilibrium on day 18 which
was maintained thereafter. However this value was markedly
different in the two breeds, the Maasai worm burden

stabilising around 224 ± 33 worms or 3.7 ± 5.95% of the
infective dose and the Merinos around 2,872 ± 334 worms or
36.6 ± 15.23% of the infective dose. In contrast, prior to day
18 worm establishment in the two breeds was very similar
(Maasai 3,906 ± 984 worms or 54.7 ± 20.32% of the infective
dose; Merinos 4,885 ± 704 worms or 60.5 ± 19.96% of the
infective dose).

The difference in establishment between the two breeds
was not significant before day 18 but very highly significant
after day 18 (P≪0.001), and the difference within each breed
before and after day 18 was significant in the Merinos (P<0.01)
and highly significant in the Maasai (P<0.001).

The mean length of worms recovered is shown in Figure 7.
The Merino worms were at all times significantly longer than
those from the Maasai sheep (P<0.01), ranging from 12mm on
day 14 to 18.5 mm on day 26 as compared with 5 and 15 mm
respectively in the Maasai.

Because only epg and not total faecal egg output was
measured on the sheep necropsied it was not possible to
estimate egg output per worm, but a very rough guide to fecund-
ity was calculated from the epg at necropsy, shown in Table 3
which indicates a slightly lower egg output per worm in the
Maasai than in the Merinos (0.677 ± 0.464 epg/worm as
compared with 0.945 ± 0.262 in the Merinos). Due to the
later patency date in the Maasai and their low or zero worm
burdens, only two Maasai were available for comparison, but

since one Maasai (which was not necropsied at this time) had
shown a positive epg on day 18 along with the Merinos, a mean
· for both breeds was taken from this point.

Circulating blood and red cell volumes calculated by the
dilution technique at the time of isotope injection are shown in
Table 4. The Merinos have a slightly larger blood and red cell
pool than the Maasai and are also slightly heavier, but breed
differences were not significant.

ii)   Experiment 2

The fluctuations in epg are shown in Figure 8. The pattern
is very similar to that obtained in the previous experiment with
the Merinos maintaining a much higher count than the Maasai. In
the Merinos patency was achieved on day 19 of infection but not
until day 22 in the Maasai which is a little earlier in both breeds
than with the Merino-adapted strain of H. contortus.

The Merino epg showed a sharp rise to 8,500 on day 35
followed by a drop to 5,500 and rising to a maximum of 10,000 epg
on day 64. In contrast, the Maasai showed a slow steady rise to
2,000 epg on day 38 which was maintained thereafter.

The Maasai epg was thus considerably higher than that
recorded in the Merino strain experiment and although the Merino
count was higher as well, the difference between the breeds was
smaller - the Maasai epg being about 25% of that of the Merinos

as compared with 4 - 12% in the previous experiment.

A comparison between the mean epg of the two breeds of sheep during infections with 'Merino-adapted' and 'Maasai-adapted' strains of H. contortus is illustrated in Figure 8a.
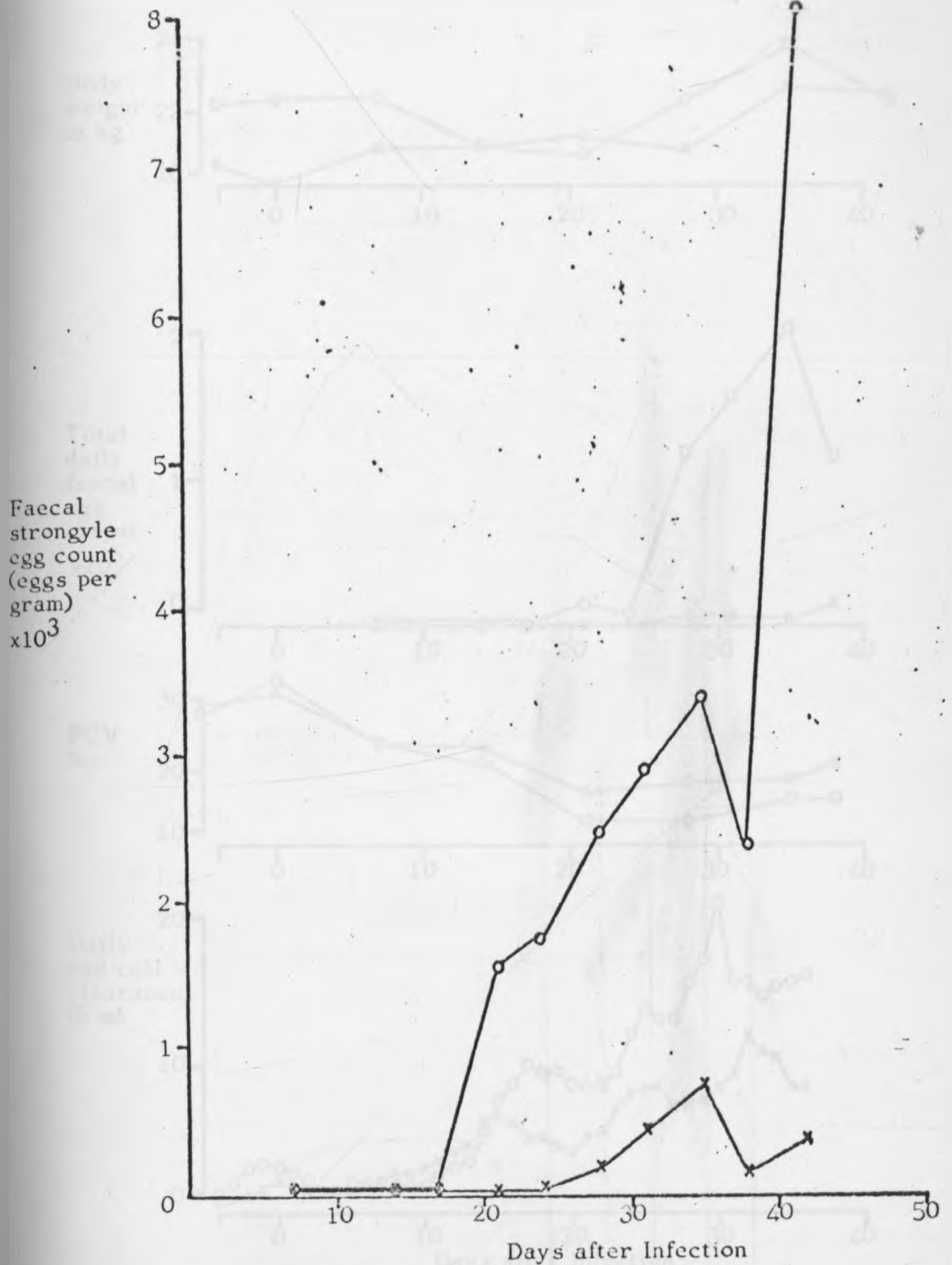
Figure 2: The mean faecal egg count (epg) of four Red Maasai (x-x) and four Merino (o-o) sheep experimentally infected with a 'Merino-adapted' strain of H. contortus

54
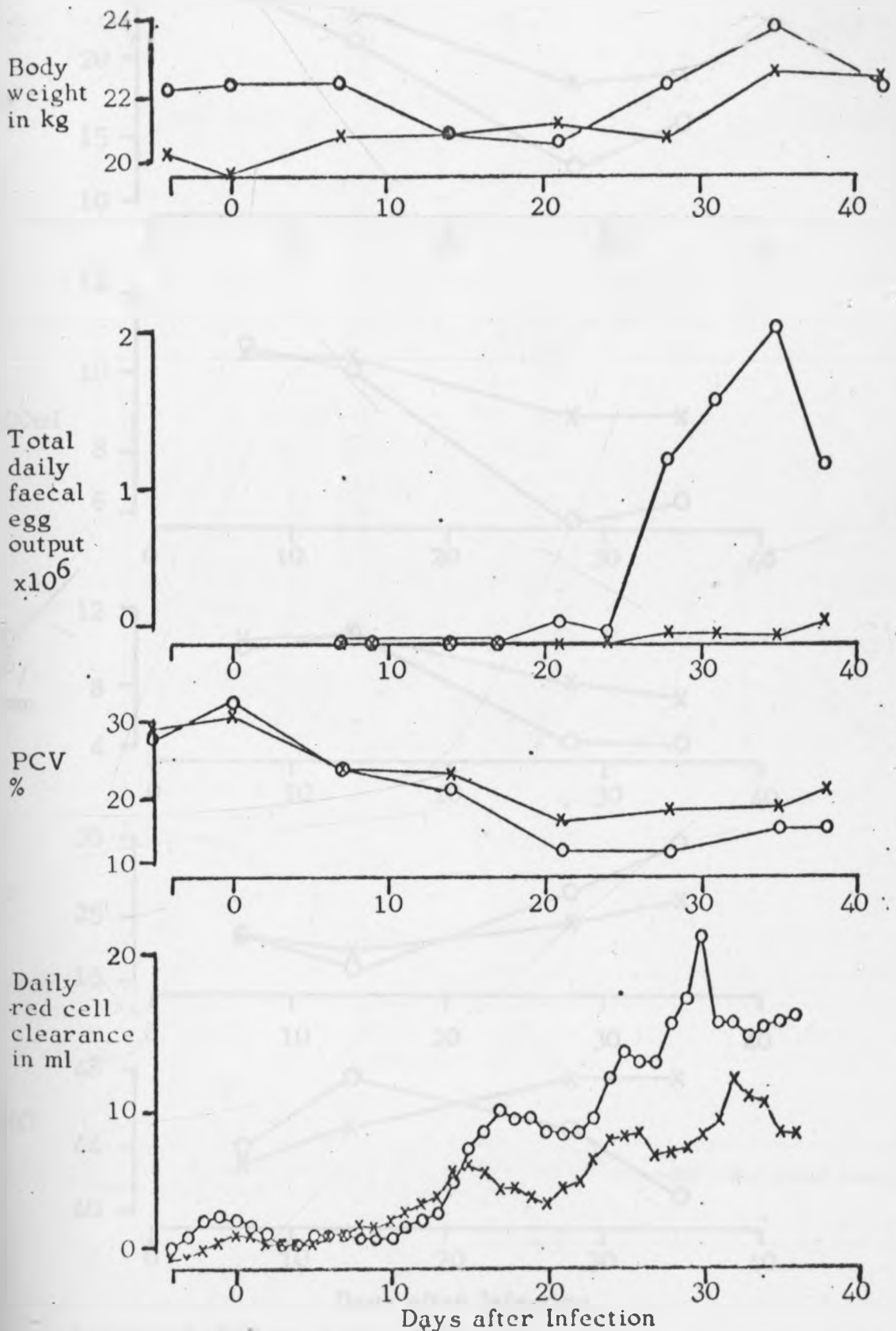
Figure 3: The mean body weight, total daily faecal egg output, haematocrit and daily loss of red cells of four Red Maasai (x-x) and four Merino (o-o) sheep experimentally infected with a 'Merino-adapted' strain of H. contortus
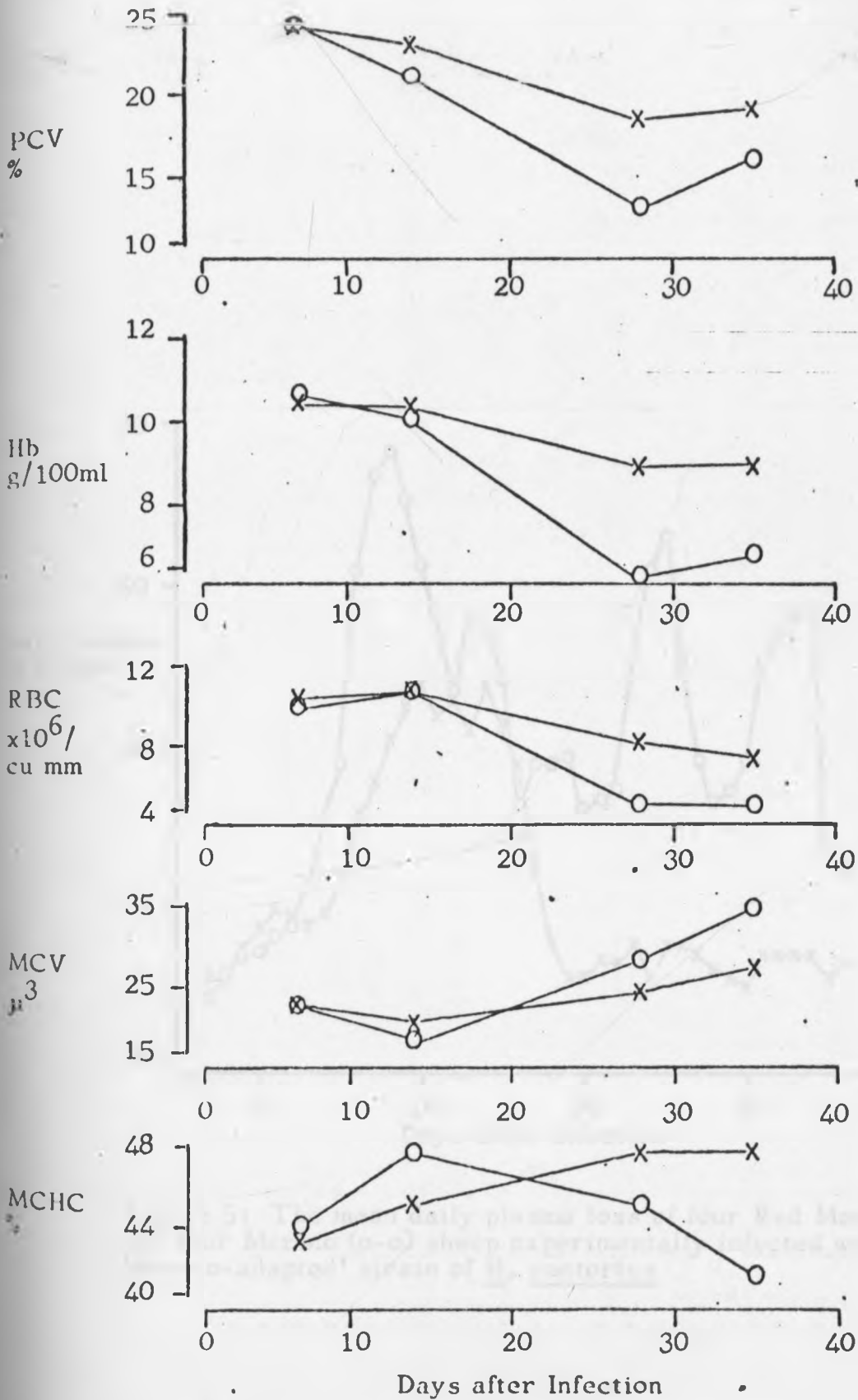
Figure 4: The mean haematocrit, haemoglobin concentration, red cell count, corpuscular volume and corpuscular haemoglobin concentration of four Red Maasai (x-x) and four Merino (o-o) sheep experimentally infected with a 'Merino-adapted' strain of H. contortus
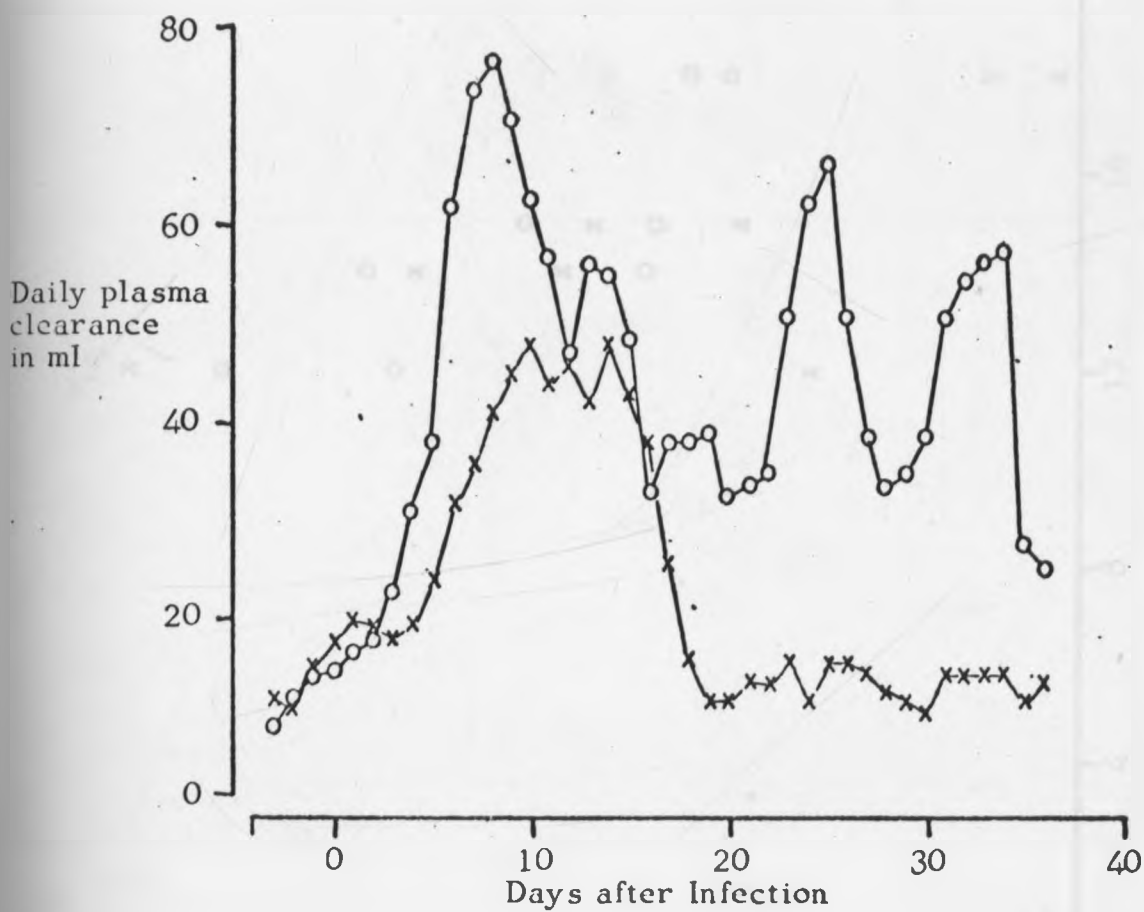
Figure 5: The mean daily plasma loss of four Red Maasai (x-x) and four Merino (o-o) sheep experimentally infected with a 'Merino-adapted' strain of H. contortus
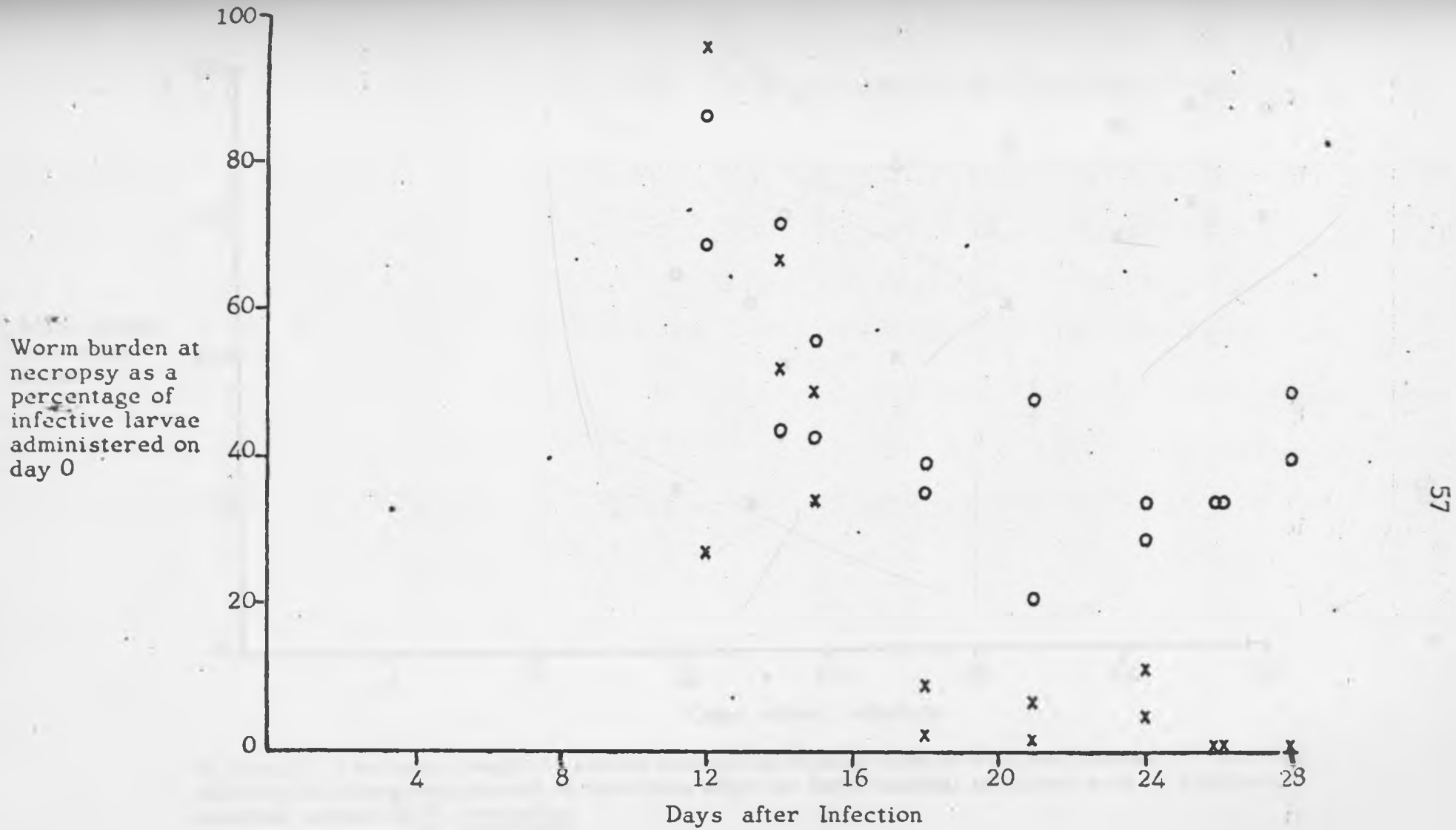
Figure 6: The mean total worm burden of two Red Maasai (x) and two Merino (o) sheep necropsied at intervals after an experimental infection with a 'Merino-adapted' strain of H. contortus
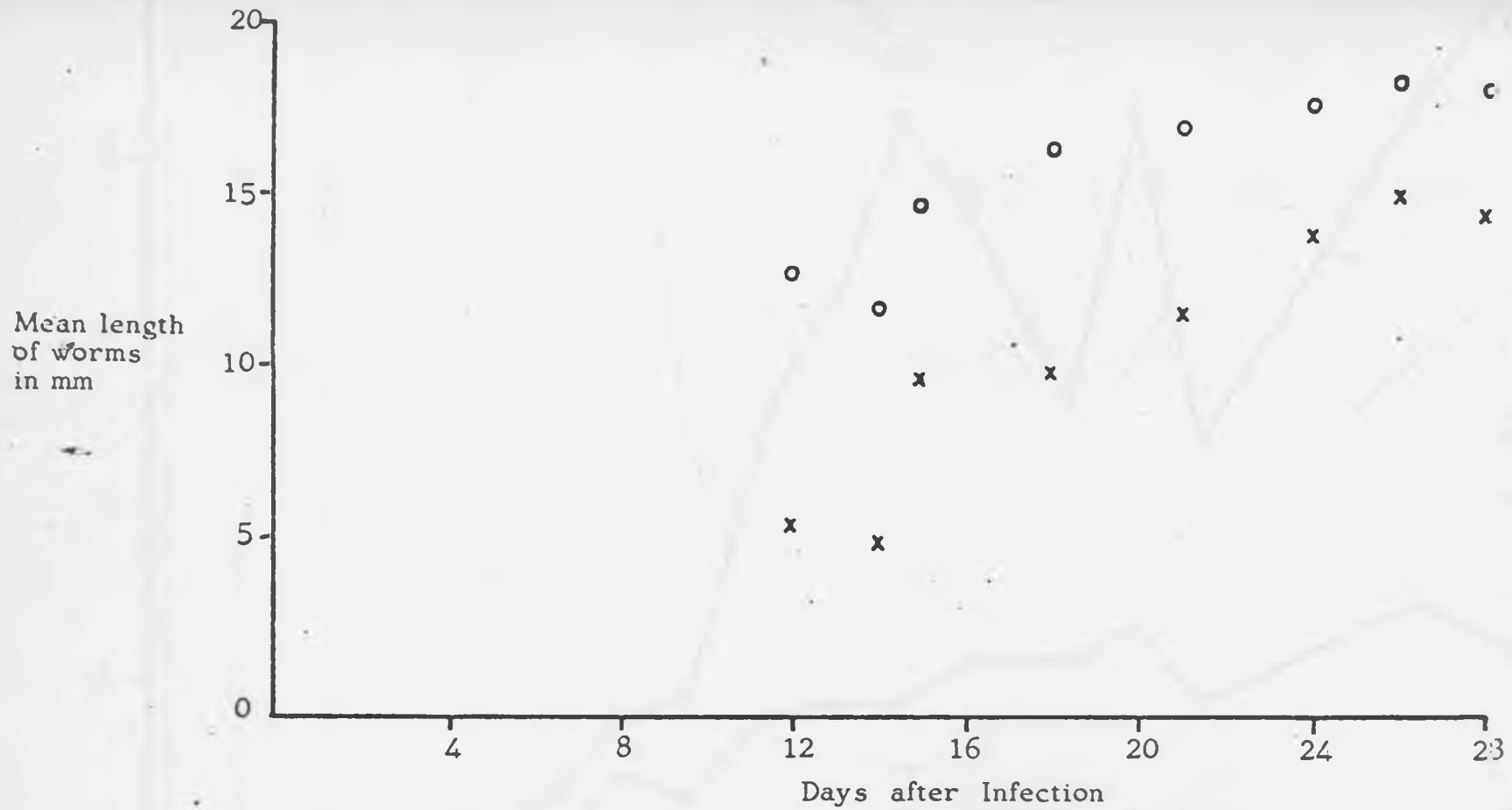
Figure 7: The mean length of worms recovered from groups of two Red Maasai (x) and two Merino (o) sheep necropsied at intervals after an experimental infection with a 'Merino-adapted' strain of H. contortus

53
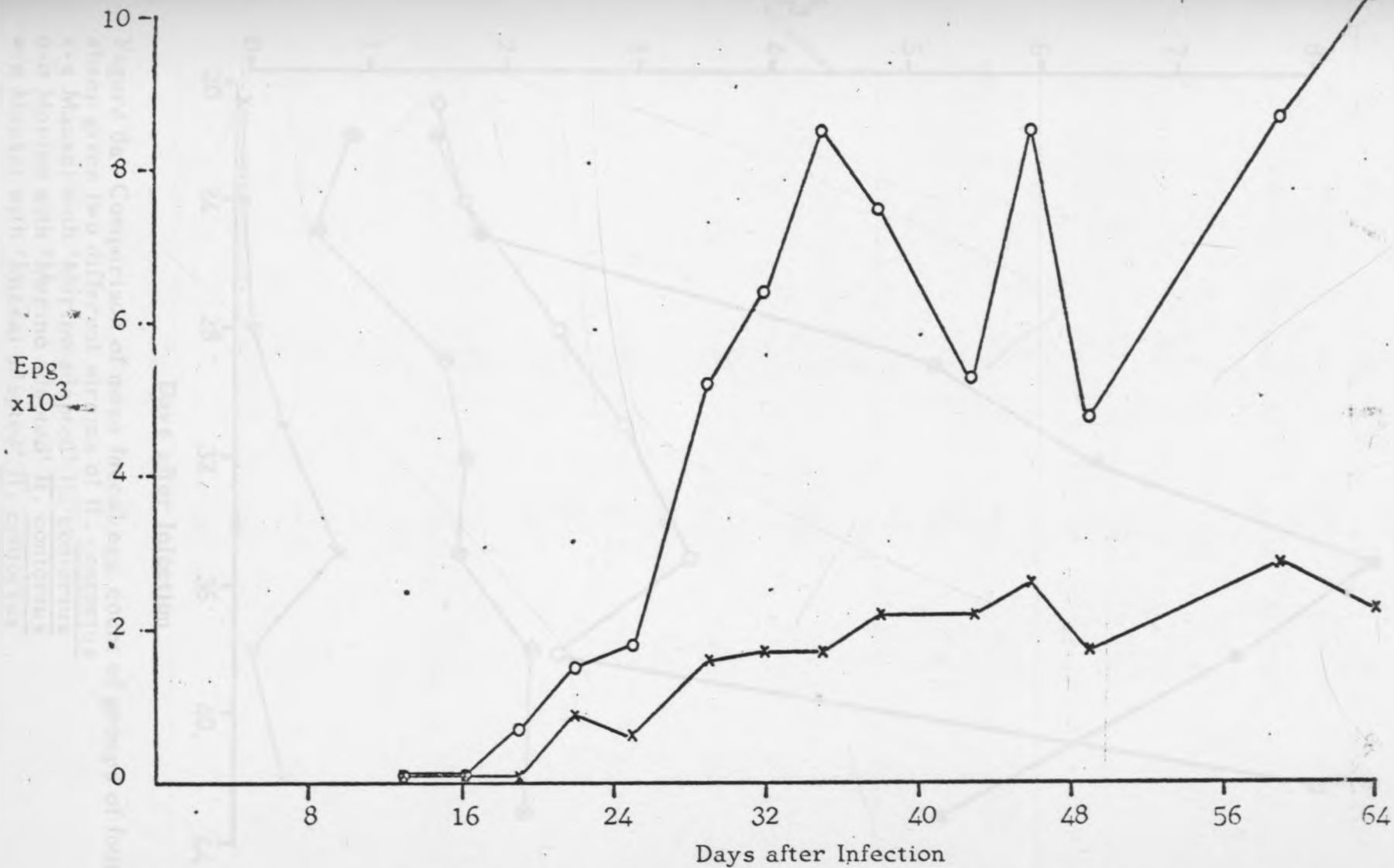
Figure 8: The mean eggs per gram of faeces of H. contortus from four Red Maasai (x-x) and four Merino (o-o) sheep given an experimental infection of 'Red Maasai-adapted' H. contortus
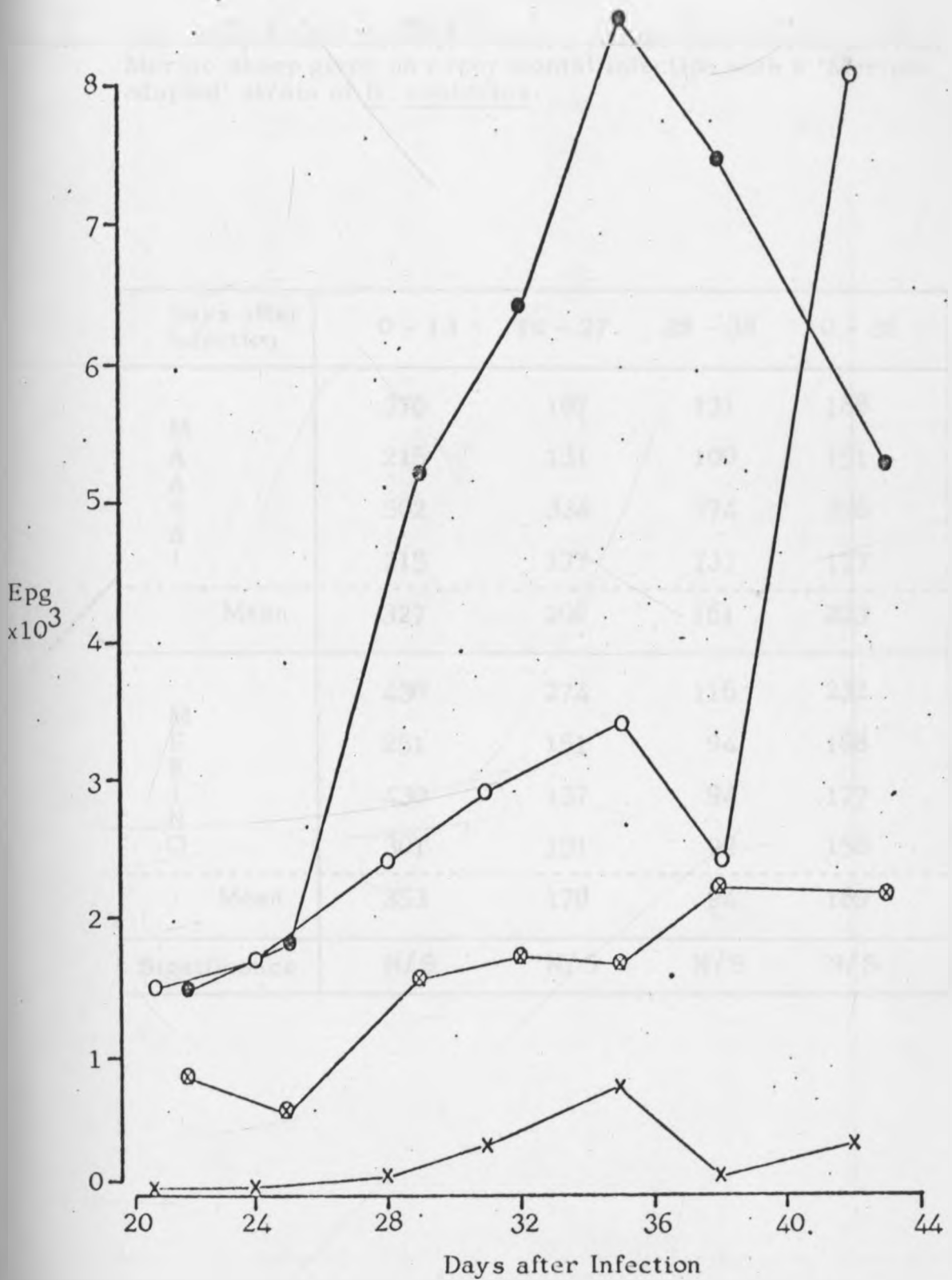
Figure 8a: Comparison of mean faecal egg count of groups of four
sheep given two different strains of H. contortus
x-x Maasai with 'Merino adapted' H. contortus
o-o Merino with 'Merino adapted' H. contortus
⊗-⊗ Maasai with 'Maasai adapted' H. contortus
●-● Merino with 'Maasai adapted' H. contortus

TABLE 1

The red cell half-life ($t_{\frac{1}{2}}$) in hours of four Red Maasai and four Merino sheep given an experimental infection with a 'Merino-adapted' strain of <u>H</u>. <u>contortus</u>.

Days after Infection	0 - 13	14 - 27	28 - 38	0 - 38
M A A S A I	376	167	131	188
	215	131	100	151
	502	334	274	376
	215	177	137	177
Mean	327	202	161	223
M E R I N O	430	274	116	232
	251	151	94	188
	430	137	94	177
	301	151	72	158
Mean	353	178	94	189
Significance	N/S	N/S	N/S	N/S

TABLE 2

The plasma half-life ($t_{\frac{1}{2}}$) in hours of four Red Maasai and four Merino sheep given an experimental infection with a 'Merino-adapted' strain of H. contortus.

Days after Infection	0 - 13	14 - 27	28 - 38	0 - 38
M A A S A I	334	301	274	301
	251	232	232	251
	251	334	376	334
	201	334	376	301
Mean	259	300	315	297
M E R I N O	301	376	232	301
	274	215	215	251
	301	301	251	274
	167	143	177	158
Mean	261	259	219	246
Significance	N/S	N/S	N/S	N/S

TABLE 3

Eggs per gram of faeces (epg), worm burden and epg/worm of Red Maasai and Merino sheep necropsied at intervals after an experimental infection with a 'Merino-adapted' strain of H. contortus.

	Sheep No	Days after Infection	epg at Necropsy	Worm Burden	epg/worm
M A A S A I	Y 82	18	0	100	0
	Y 87	18	0	600	0
	Y 92	21	0	100	0
	Y 98	21	0	400	0
	Y 81	24	800	600	1.333
	Y 85	24	600	220	2.727
	PYNE	26	0	0	0
	M 58	26	0	0	0
	Y 91	28	0	0	0
Mean			156	224	0.677
			±104	± 83	±0.464
M E R I N O	T 209	18	3,800	2,210	1.719
	B 154	18	1,800	2,000	0.900
	B NN	21	1,900	2,050	0.927
	B 158	21	1,700	1,200	1.417
	A 260	24	1,400	2,880	0.486
	B 41	24	5,600	2,020	2.772
	G 20	26	600	3,790	0.158
	G 29	26	300	3,490	0.086
	M132	28	2,000	5,280	0.379
	M134	28	2,300	3,800	0.605
Mean			2,140	2,872	0.945
			± 483	± 384	±0.262

TABLE 4

Circulating blood and red cell volumes (in ml) of four Red Maasai and four Merino sheep before infection with a 'Merino-adapted' strain of H. contortus shown with venous haematocrit and body weight for comparison.

		Body Weight in kg	PCV	Blood Volume	Red Cell Volume	Red Cell Vol/kg Body Wt
MAASAI		22.5	29	1,230.00	356.70	15.85
		18.5	27	910.75	245.90	13.29
		24.0	28	1,325.10	371.03	15.46
		17.5	31	944.81	292.89	16.74
	Mean	20.6 ±1.56	28.8 ±0.85	1,102.67 ±103.05	316.63 ±29.06	15.34 ±0.73
MERINO		22.5	29	1,502.78	435.81	19.37
		25.0	31	1,317.87	408.54	16.34
		23.5	31	1,352.99	419.43	17.85
		18.0	20	1,264.55	252.91	14.05
	Mean	22.3 ±1.51	27.8 ±2.63	1,359.55 ±51.09	379.17 ±42.46	16.90 ±1.13
Significance		N/S	N/S	N/S	N/S	N/S

DISCUSSION

The results of these experiments indicate a very marked difference in the response of Red Maasai and Merino sheep to an experimental infection with Haemonchus contortus and confirm the earlier field observations of Preston and Allonby (in press).

This difference is reflected not only in the pathogenesis of the disease but also in the actual numbers of worms present and in their size and possibly fecundity as well and is not related to the strain of parasite used since the response of both breeds was comparable in infections involving either 'Maasai-adapted' or 'Merino-adapted' worms.

The difference in the pathophysiology of the disease in Red Maasai and Merino sheep can be related directly to worm numbers and is not due to some greater ability on the part of the Maasai sheep to resist or compensate for the effects of the disease by an increased erythropoietic response or by a larger initial blood volume. In fact, in the sheep used in the first experiment the Merinos were apparently better placed to compensate for blood loss, having a higher initial blood and red cell volume than their Maasai counterparts.

This observation is in agreement with that of Altaif (1975) in Scottish Blackface and Finn Dorset sheep that resistance to the pathophysiological effects of infection with H. contortus is of minor importance when compared with the differences in worm

numbers between the two breeds to which the pathophysiological
effects could be closely related.

The difference in worm burden between the Red Maasai and
Merino sheep did not occur until 16 to 18 days after infection
when both breeds experienced a rapid and significant drop in
worm numbers. Until this time establishment and retention of the
infective larvae was comparable in the Maasai and Merino sheep
with both breeds harbouring 50 - 60% of the larvae administered
(Maasai 54.7 ± 20.32%, Merino 60.5 ± 19.96%) but after day 18
this had dropped to 4% (3.7 ± 5.95%) in the Maasai but to only 37%
(36.6 ± 15.23%) in the Merinos. No appreciable loss of worms
occurred after this time.

Altaif (1975) reported a very similar pattern of expulsion in
Scottish Blackface and Finn Dorset sheep and suggested that
resistance to infection was not expressed until the fourth to fifth-
stage larval moult 9 - 11 days after infection (Soulsby, 1965)
had occurred and then increased in intensity over the following
6 - 8 days. This coincides exactly with the results of this trial
in which no breed differences were evident until their dramatic
appearance between days 16 and 18 when worm expulsion
occurred.

As a direct result of this difference in worm numbers, there
was a marked separation in all indices of disease after day 16 with
the Merinos presenting a typical picture of haemonchosis namely
hypercatabolism of plasma (up to 77 ml per day) and a high blood

loss (up to 21 ml of red cells per day) into the gastro-intestinal
tract due to the voracious feeding activities of large numbers of
worms and a consequent drop in PCV and Hb concentration, while
the Maasai showed little clinical evidence of disease.

The blood loss results in the development of an anaemia which
can be reproduced experimentally by phlebotomy (Fourie, 1931;
Andrews, 1942) and is due solely to haemorrhage and not to some
inhibitory effect of the parasites upon erythropoiesis (Baker and
Douglas, 1966). In fact, the very opposite occurs, as shown
by Dargie and Allonby (1975) in that the erythropoietic system is
markedly stimulated by the haemorrhage and a stage is reached
where the PCV may remain steady or even rise in the presence
of increasing haemorrhage. This can be seen in Figure 4 where
on day 28 the PCV, Hb and RBC levelled out and increased
slightly after day 35 while red cell loss (Figure 3) reached its
maximum on day 30 in the Merinos and on day 32 in the Maasai.

The period up to day 28, illustrated in Figure 4 represents
a lag phase in the response of the sheep to haemorrhage during
which PCV, Hb and RBC fall due to the feeding activities of the
fourth-stage and young fifth-stage larvae. During this period
the erythropoietic system is stimulated but is unable to respond
immediately, giving rise to a dangerous lag period during which
blood lost into the gut is not replaced. Since this phase occurs
prior to patency, the disease frequently goes undiagnosed and
therefore untreated. Fatal prepatent cases of haemonchosis
have been reported experimentally (Andrews, 1942) and are

probably common in natural infections but not recognised or
only diagnosed at necropsy (Urquhart, 1964).

The period after day 28 represents the second stage of
infection as described by Dargie and Allonby (1975) during
which the stimulated erythropoietic system is able to compensate
for blood loss and haematological indices may remain steady or
even increase. When the worm burden is large and thus the
blood loss is considerable, haemopoiesis may be operating at
the expense of the body's iron stores since relatively small
quantities of iron are resorbed from the gut as shown by studies
with ^{59}Fe labelled blood (Altaif, 1975; Dargie and Allonby, 1975).
This imbalance leads to the final stage which is heralded by a
sharp drop in PCV, Hb, RBC and serum iron due to exhaustion
of the body's iron stores and is normally rapidly fatal even when
blood loss remains unchanged. This stage was not reached in
this study due to the termination of infection before the onset of
haemopoietic exhaustion.

The anaemia produced is initially normocytic and
normochromic (Allonby and Urquhart, 1975) but the rapid
stimulation of the erythron leads to an increase in the MCV and
a fall in the MCHC due to the presence of large numbers of
reticulocytes in the circulation. This is illustrated in Figure 4
where after day 14 the steady decline in PCV, Hb and RBC is
complemented by a slight increase in MCV and a fall in the MCHC
in the Merinos. During the final stage of haemonchosis
exhaustion of the erythropoietic system may give rise to a

microcytic, hypochromic anaemia (Baker and Douglas, 1966).

As a natural corollary of prolonged blood loss, weight loss is usually a marked symptom of haemonchosis. However, diet is known to be important in influencing the pathogenesis of an infection with H. contortus (Nagahata, Fujita and Ikegaya, 1941; Whitlock, Callaway and Jepperson, 1943; Whitlock, 1949; Laurence et al, 1951; Djafar, 1962; Kates, Allen and Wilson, 1962) so to reduce any dietary effects on pathogenesis in this experiment, the sheep were maintained on a high quality diet given to them ad lib. The result of this is that no weight loss was recorded during the infection although the Merino sheep were experiencing a considerable protein drain due to haemorrhage which on a less nutritious diet would have been at the expense of fat stores and been translated into a weight loss.

It has already been noted that the major difference between the Red Maasai and Merino sheep was in worm numbers and that the pathophysiology of the infection can be related entirely to this factor. However, further differences were noted in the actual worms recovered from the two breeds.

Many authors have reported differences in the physiology, fecundity and size of worms recovered from resistant hosts and these are generally believed to be the result of an immune response by the host (Silverman and Patterson, 1960; Urquhart Jarrett and Mulligan, 1962; Michel, 1963; Dineen and Wagland, 1966a; 1966b; Ogilvie and Jones, 1971; 1973; Ogilvie and Love,

1974).

Incubation in vitro of H. contortus (Silverman and
Patterson, 1960) and Nippostrongylus brasiliensis (Ogilvie and
Jones, 1971; 1973; Ogilvie and Love, 1974) worms in immune
serum has resulted in precipitate formation at the worms'
excretory pore and rectal sphincter and degenerative changes
in the gut. Incubation of adult N. brasiliensis worms in immune
serum adversely affects their metabolism as measured by ^{32}P
uptake (Ogilvie and Jones, 1973; Ogilvie and Love, 1974) and
incubation of the infective larvae of the other species in
immune serum reduces their infectivity (Urquhart, Jarrett and
Mulligan, 1962). It seems likely that worms face similar adverse
conditions in vivo and Nippostrongylus worms have been recov-
ered from resistant hosts with very similar degenerative changes
to those seen in vitro (Ogilvie and Jones 1973; Ogilvie and Love,
1974).

The very marked stunting of worms recovered from Maasai
sheep, shown in Figure 7, is thus an indication that immune
phenomena have been operative and their effect upon the worms
has been to interfere with their normal growth pattern.
Stunting of adult worms in resistant hosts has also been reported
in Nippostrongylus by Chandler (1932), Taliaferro and Sarles
(1939) and Ogilvie and Jones (1973) and in Haemonchus by
Roberts (1957), Silverman and Patterson (1960), Christie and
Brambell (1967), Christie, Brambell and Charleston (1964) and
Radhakrishnan et al (1972). Inhibition of worm growth is

generally considered to be due to an immune reaction by the host and may well affect the pathogenicity of the infection, but because necropsies were not conducted on the sheep whose blood loss was monitored, it was not possible to relate blood loss to worm size.

The influence of immunity on the fecundity of parasites has important epidemiological implications and may operate by either delaying patency or reducing the egg output of the parasites, both of which reduce the number of infective larvae available for reinfection. Silverman and Patterson (1960) reported that patency of an infection of H. contortus occurred between 12 and 15 days after infection in susceptible lambs but not until day 16 to 24 in older sheep. Likewise, Radhakrishnan, Bradley and Loggins (1972) observed patency of H. contortus on day 17 in Rambouillet lambs but not until day 20 in the more resistant Florida Native lambs. The results reported here are generally in agreement with this, although patency was a little later in both groups, namely 21 days (Merino) and 28 days (Maasai) respectively after infection.

A marked reduction in egg production by Nippostrongylus worms in resistant rats was reported as early as 1932 by Chandler and later confirmed by Taliaferro and Sarles (1939) and Ogilvie and Love (1974), and has since been reported in other nematodes including H. contortus.

Roberts (1957), working with experimental infections of

H. placei in calves was able to induce a strong resistance to
infection and examination of the worm burden of resistant
calves showed that it consisted of relatively few adult worms
most of which were undersized and that the females contained
very few or no eggs at all. The epg in resistant calves was
similarly very low and led Roberts to observe that epg,
although a good index of worm burden in susceptible calves is
of little value in resistant calves. Dineen and Wagland (1966a)
reported a decreased egg output per female H. contortus worm
in previously worm-free lambs undergoing a second challenge
infection and in sensitised lambs compared with worm-free
controls (Wagland and Dineen, 1967).

The limited information available in this experiment suggests
that the fecundity of H. contortus in the Maasai breed (0.677
epg/worm) is lower than that in the Merinos (0.945 epg/worm),
but both of these values are lower than that calculated by
Cvetkovíc, Lepojev and Lalíc (1975) of 1.7 - 3.0 H. contortus
eggs per gram of faeces. This index was however based on an
infection in 8-month old lambs while the sheep used here were
all adults with experience of infection from birth and may
therefore be expected to have acquired some degree of immunity
regardless of breed.

It is clear from the results of this trial that a very marked
difference in response to infection with H. contortus occurs in
Red Maasai and Merino sheep as judged by worm numbers
recovered, egg output in the faeces and pathophysiological

parameters

    The occurrence of such factors as stunting of the worms and impairment of their reproductive potential suggests that immune phenomena are operative in the host-parasite relationship. However the nature of the most critical part of this relationship, namely worm expulsion 16 - 18 days after infection, remains obscure. It is feasible that the worms encounter some physiological set-back at the time they reach maturity, related possibly to their increased demand for blood or due to overcrowding or perhaps by eliciting a non-specific allergic reaction in the mucosa which results in the expulsion of the majority of the worms.

    Alternatively, worm expulsion may be brought about by an immune reaction on the part of the host acting specifically on the worms as a result of antigenic sensitisation. If this is the case the timing and degree of this reaction may be expected to differ in a primary and secondary infection, the primary infection sensitising the host and eliciting a primary response while subsequent infections cause a much faster and stronger secondary response.

SUMMARY

Four Red Maasai and four Merino sheep were given an experimental infection of Haemonchus contortus at 350 L3/kg body weight. The sheep used were adult wethers reared on pastures known to be infected with H. contortus. Two experiments were conducted using first a 'Merino-adapted' strain of worm and then a 'Maasai-adapted' strain. No difference was observed in the egg counts obtained from the two worm strains so breed differences observed were concluded to be independent of worm strain.

Response to infection was monitored using ^{51}Cr labelled erythrocytes, ^{125}I labelled plasma, haematocrit, total faecal egg output and body weight changes. Worm burdens were obtained from 16 sheep killed at intervals from 12 - 28 days after infection.

No breed difference was observed before day 16 - 18 when both breeds underwent an expulsion of worms which resulted in a reduction to 4% of larvae administered in the Maasai but to only 37% in the Merinos. From this time onwards marked breed differences were noted in red cell and plasma loss into the gut, red cell and plasma half-life, PCV and other haematological indices and epg, all of which could be directly related to the difference in worm burden between the two breeds.

A difference was also noted in the size of worms recovered, which were smaller in the Maasai breed throughout, in the length

of the prepatent period and there was limited evidence of a reduced fecundity in worms from Maasai sheep. These differences suggest that immune phenomena are operative, particularly in the Maasai sheep.

THE INFLUENCE OF BREED ON THE RESPONSE OF SHEEP TO PRIMARY INFECTION WITH HAEMONCHUS CONTORTUS

The results reported in the previous section of this thesis confirm that a very marked difference occurs in the resistance of adult Red Maasai and Merino sheep reared on contaminated pastures to infection with H. contortus. The resistance of these sheep which appears to be at least partly immunological, may be acquired by experience of infection or it may be genetically determined and operative from birth. This is an important consideration in view of the fact that newborn lambs may be exposed to heavy infections of Haemonchus in the field.

The majority of lambs are born at the start of the rainy season when pasture growth is at its maximum, but this is also the time of maximum pasture contamination with infective larvae of trichostrongylid worms. The high humidity during the rains favours parasite survival on the pasture (Dinnik and Dinnik, 1961) and lambing is known to be associated with a decreased immunity to parasites by the ewes and a consequent increase in egg output by the parasites (Connan, 1968). Hence neonate lambs with no acquired immunity to resist infection are exposed to high challenge levels as soon as they are weaned. Furthermore, in many breeds of sheep a long period of immunological unresponsiveness is known to exist during which the lambs are unable to resist infection by H. contortus although able to

respond to other less complex antigens (Urquhart, 1964). This
is a potentially lethal combination, yet the majority of lambs
normally survive and grow to maturity.

In order to avoid the added complication of the immunologic-
ally unresponsive period which in Merinos is known to exist
until about 7 months of age (Lopez and Urquhart, 1967), Red
Maasai and Merino lambs were reared indoors, free from
infection to 10 - 13 months of age and then experimentally
infected with H. contortus in order to monitor their response
to primary infection at an age when they would be immunologic-
ally mature.

METHODS AND MATERIALS

Four Red Maasai and four Merino lambs which had been reared worm-free were confined in metabolism cages and given a primary experimental infection of H. contortus at 250 L3/kg. Their response to infection was monitored using ^{51}Cr labelled autologous red cells given 5 days before infection, venous haematocrit, epg and weekly weight changes. Sampling was continued for 45 days from the day of labelling.

Forty-eight days later the lambs were given more autologous ^{51}Cr labelled red cells and challenged with H. contortus at 350 L3/kg 12 days later. Their response to challenge was monitored in the same way for 40 days.

At the end of each isotopic run, all samples were counted in a well-type scintillation counter (Packard) on the same day and calculations made to estimate blood loss.

i) Experimental Animals

Since 8 worm-free male lambs were not available, two males and two females from each breed were used. These were all of haemoglobin type B, aged 10 - 13 months at the start of the experiment, which had been born and reared indoors under worm-free conditions at the Veterinary Research Laboratories, Kabete. They had been weaned at 4 months of age and the tails of the Merinos docked and the males castrated. No inoculations were

performed on any lambs.

The lambs were drenched twice with thiabendazole
('Thibenzole' - Merck, Sharp and Dohme, Australia, Pty. Ltd.)
at 100 mg/kg 28 days and again 2 days before the start of the
experiment and placed in metabolism cages and the males fitted
with faecal collecting bags one month before infection.

The lambs were maintained on Rhodes hay and lucerne
supplemented with concentrates ad lib and their cages were
cleaned thoroughly every other day.

ii) Parasites

The Merino-adapted strain of H. contortus was used in both
the primary infection and the challenge. This was collected
from Merino sheep at the National Animal Husbandry Research
Station, Naivasha and passaged through a Merino wether at the
Veterinary Research Laboratories, Kabete.

iii) Parasitological Techniques

Culture and administration of infective larvae and faecal egg
counts were conducted as described in the previous section.
Necropsies were not performed.

iv) Labelling of Erythrocytes with ^{51}Cr

This was performed as before at $100 \,\mu$ Ci/ml packed red cells. Labelled cells were injected back into their donors via a jugular catheter and a blood sample taken from the opposite vein exactly 10 minutes later.

v) Sampling Methods

Daily blood samples were collected and a 1 ml sample pipetted into 9 ml 0.05% NaOH as already described.

Urine and faeces were collected, measured and sampled as before except in the females, whose faeces were collected in buckets placed beneath the cages where slight contamination with urine was unavoidable.

vi) Blood analyses

Venous haematocrit was estimated by the micro-haematocrit method, other blood analyses were not performed.

vii) Calculations

Estimation of daily red cell loss into the gut was made as before and red cell half-life calculated from a semilogarithmic plot of percentage activity against time. Circulating blood and red cell volumes were calculated by the dilution principle.

RESULTS

The epg for the whole period of infection and challenge is shown in Figure 9. The difference between the two breeds became evident within 15 days of patency with the Maasai maintaining a mean epg (590 ± 105.1) which is only 12% of that of the Merinos (4,813 ± 691.2). The mean Maasai epg can be divided into three sections, the first up to day 60 fluctuating around a mean of 1,032 ± 181.2 followed by a steady decline until challenge after which a low mean of 125 ± 43.5 was established.

There was no difference between the breeds in the day on which the infection became patent. In both breeds, one lamb had detectable worm eggs on day 21, two on day 22 and all four by day 25. This is summarised in Table 5.

In Figures 10 and 11 faecal egg output is shown in relation to body weight changes, PCV and daily red cell loss for the primary infection and the challenge infection 100 days later. The total faecal egg output follows the same trend as the epg for these periods shown in Figure 9.

Both breeds gained rather than lost weight during the course of the primary infection, in the Merinos this was statistic- ally significant (P<0.01) but there was no significant breed difference in the amount of weight gained.

Both breeds experienced a fall in PCV during the first 40 days

of infection with the Maasai PCV falling from a mean of
35.3 ± 2.78% to 21.9 ± 2.10% and a similar fall in the Merinos
from 28.0 ± 1.58% to 17.8 ± 1.93%. However 12 days before
challenge both breeds had re-established their prepatent means
(Maasai 31.5 ± 0.91% and Merinos 28.0 ± 1.26%) and by the end
of sampling 26 days later, the PCV in both breeds had fallen to
a joint mean value of 26%.

There was no significant difference in the mean PCV of the
two breeds prior to day 26 of the primary infection (Maasai
30.5 ± 2.17%; Merino 25.8 ± 1.67%), but after day 26 the
difference was significant(P<0.01) (Maasai 23.3 ± 0.86%; Merino
17.8 ± 1.20%). The fall in PCV was significant only in the
Merinos (P<0.05)

Breed differences in red cell loss were not apparent before
day 26 but after this the Merino red cell loss was almost double
that of the Maasai. This increase was statistically significant
only in the Merinos (P<0.05). From day 26 a clear separation
occurred between the breeds with the Maasai experiencing a
mean red cell loss of 8.7 ± 1.66 ml per day compared with a
Merino mean of 16.7 ± 4.45 ml per day. This is summarised
in Tables 6 and 7. By the time of challenge, the red cell loss in
the two breeds had separated into two distinct levels which were
closely correlated with the epg and thus worm burden. The
effect of challenge was to cause a small fall both in red cell loss
and epg which was followed 15 days later by a rise in both epg
and red cell loss which was particularly marked in the Merinos.

After challenge the mean Merino red cell loss was
7.8 ± 2.98 ml/day while that of the Maasai was 3.0 ± 0.67 ml/day.
In the Maasai the mean red cell loss after challenge was slightly
less than that during the first 26 days of primary infection
(4.2 ± 0.58 ml/day) but considerably less than that after day
26 (8.7 ± 1.66 ml/day). In the Merinos the daily red cell loss
after challenge (7.8 ± 2.98 ml/day) was double that during the
first 26 days of the primary infection (3.5 ± 0.57 ml/day) and
half that after day 26 of the primary infection (16.7 ± 4.45 ml/day).
These differences were however not statistically significant.

The red cell half-life ($t_{\frac{1}{2}}$) shown in Tables 8 and 9 is a further
reflection of the difference in red cell loss between the two breeds
with the Maasai red cell $t_{\frac{1}{2}}$ at all times greater than that of the
mean Merino value and the difference between them increasing
during the course of the infection.

Since necropsies were not conducted, it was not possible to
estimate red cell loss per worm or egg output per worm. Table 10
however shows the relationship between egg output and red cell
loss. The average number of H. contortus eggs produced per ml
of red cells lost is lower in the Maasai than the Merinos and
upon challenge was further markedly reduced from 84.98 to 35.87,
while the mean Merino value remained unchanged (120.91 to 120.22).

Circulating blood and red cell volumes calculated by the
dilution technique at the time of isotope injection are shown in
Tables 11 and 12. With the exception of the blood volume before

primary infection, in each case the Maasai value is slightly
larger than that of the Merinos, but breed differences were in
no case significant. Significant differences occurred between
primary infection and challenge in the Maasai PCV which fell
from a mean of 42% to 32% and in the mean Merino weight which
increased and PCV, red cell volume and red cell volume per
kilogram body weight which all decreased between primary
infection and challenge.

Although mean Merino values are shown throughout this
experiment, considerable variation existed between the individual
lambs with the two females being considerably more resistant to
infection as judged by epg and red cell loss than the two males.
However in view of the small sample size, a mean value for the
Merino breed has been calculated. This difference was not
considered to be sex-linked but to be an example of the wide
variation which exists in the response of Merino sheep to
infection with H. contortus. These same Merino lambs were
used in a high infection level experiment described in the following
section, and their response to high infection followed the same
pattern as that described in this section. No such difference
existed in the Maasai lambs even though both females lambed
during the course of the experiment - one on the day before
infection and the other 48 days later.
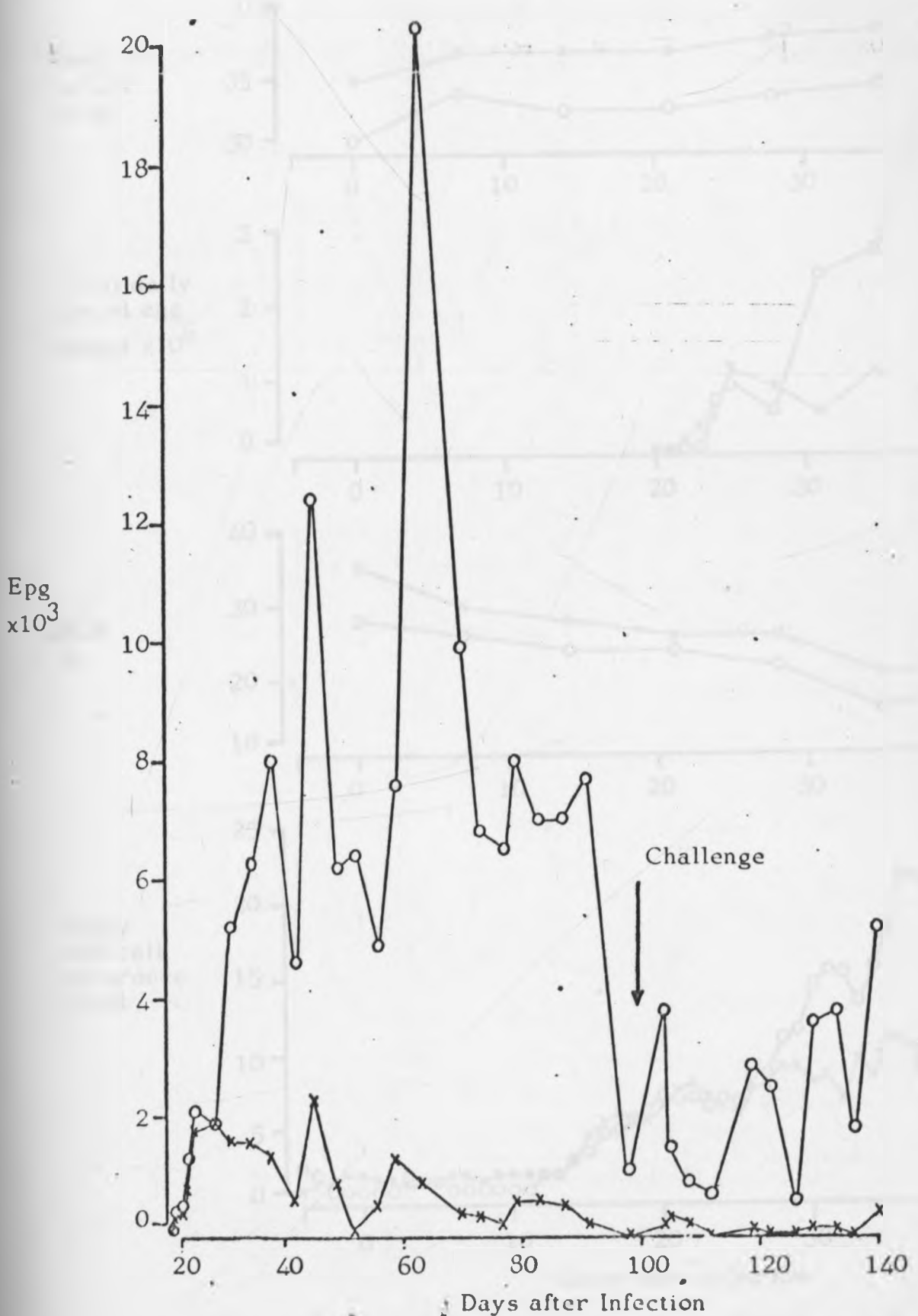
Figure 9: The mean faecal egg count (epg) of four Red Maasai (x-x) and four Merino (o-o) lambs given a primary experimental infection of H. contortus and challenged 100 days later
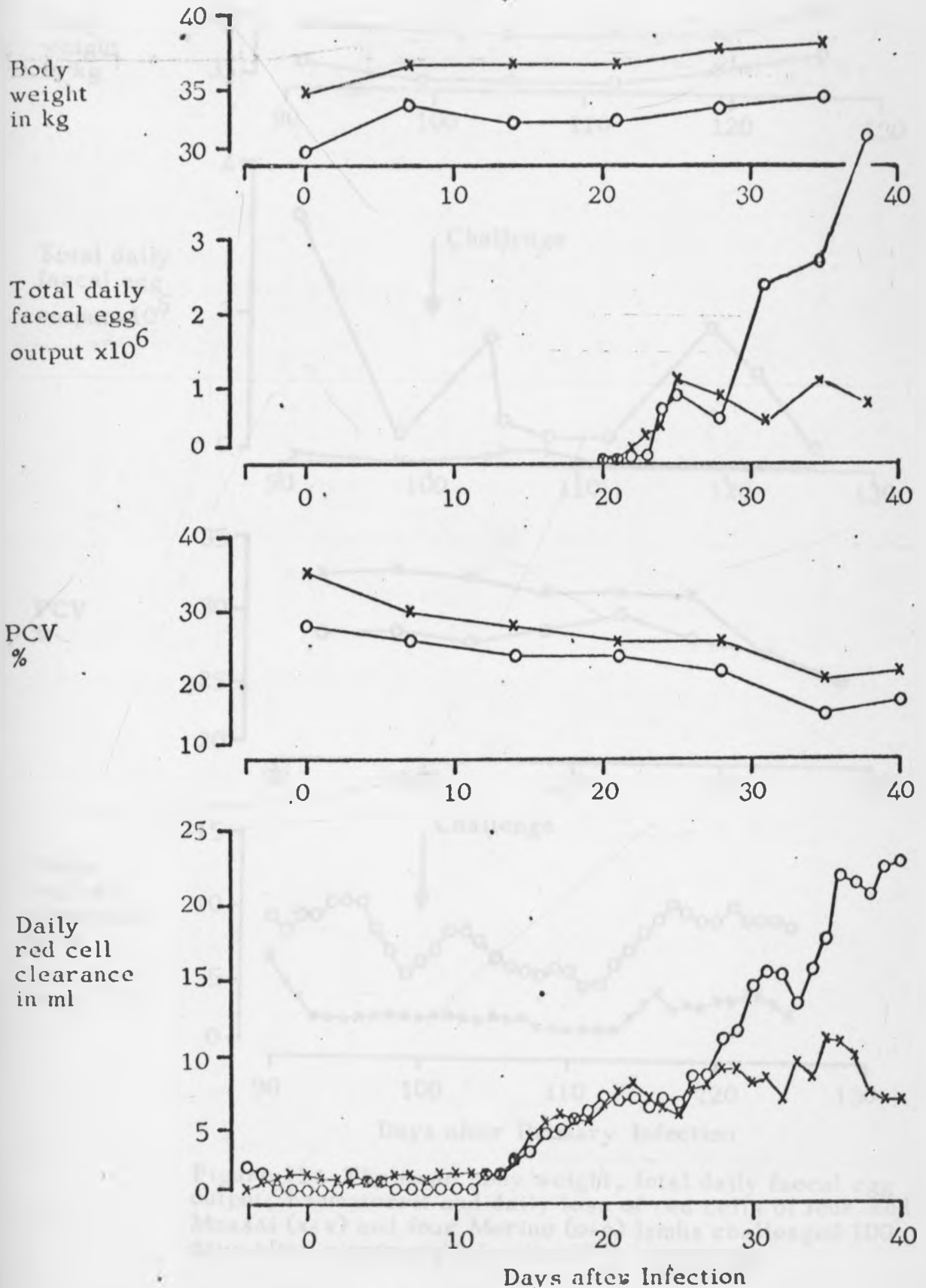
Figure 10: The mean body weight, total daily faecal egg output, haematocrit and daily loss of red cells of four Red Maasai (x-x) and four Merino (o-o) lambs given a primary experimental infection of H. contortus
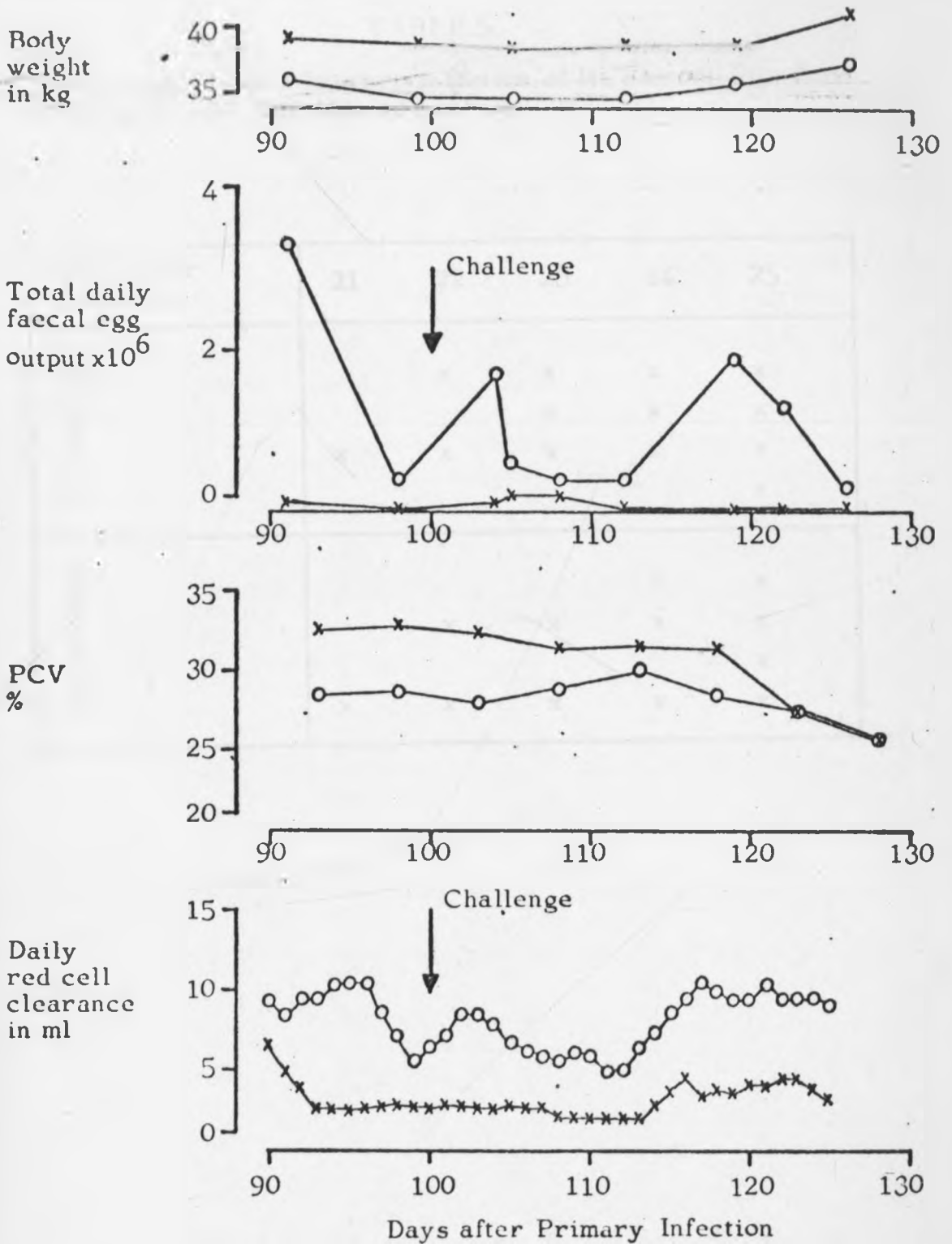
Figure 11: The mean body weight, total daily faecal egg output, haematocrit and daily loss of red cells of four Red Maasai (x-x) and four Merino (o-o) lambs challenged 100 days after a primary infection of H. contortus

TABLE 5

Date of patency of a primary infection of H. contortus in four Red Maasai and four Merino lambs.

Days after Infection	21	22	23	24	25
M A A S A I		x	x	x	x
			x	x	x
	x	x	x	x	x
					x
M E R I N O				x	x
		x	x	x	x
					x
	x	x	x	x	x

TABLE 6

The mean daily red cell loss (in ml ± SE) of four Red Maasai and four Merino lambs before and after the 26th day of a primary infection with H. contortus and after challenge 100 days later.

	Days 0 - 26	Days 27 - 40	Challenge
Red Maasai	4.2 ± 0.58	8.7 ± 1.66	3.0 ± 0.67
Merino	3.5 ± 0.57	16.7 ± 4.45	7.8 ± 2.98

TABLE 7

Statistical comparison (t-test) of mean daily red cell loss of four Red Maasai and four Merino lambs before (1) and after (2) the 26th day of a primary infection with H. contortus and after challenge (3) 100 days later.

	1 & 2	2 & 3	1 & 3
Red Maasai	N/S	N/S	N/S
Merino	P<0.05	N/S	N/S
	Days 0 - 26	Days 27 - 40	Challenge
Red Maasai v Merino	N/S	N/S	N/S

TABLE 8

The red cell half-life ($t_{\frac{1}{2}}$) in hours of four Red Maasai and four Merino lambs given an experimental primary infection of H. contortus.

Days after Infection	0 - 13	14 - 27	28 - 40	0 - 40
M A A S A I	376	301	201	274
	430	251	188	274
	376	215	188	232
	376	502	251	376
Mean	390	317	207	289
M E R I N O	502	274	89	215
	430	215	137	232
	274	301	72	201
	301	201	201	215
Mean	377	248	125	216
Significance	N/S	N/S	$P < 0.05$	N/S

TABLE 9

The red cell half-life ($t_{\frac{1}{2}}$) in hours of four Red Maasai and four Merino lambs challenged 100 days after a primary infection of H. contortus. The first column represents the period 8 days before challenge to 6 days after challenge.

Days after Challenge	(-8) - 6	7 - 20	21 - 28	0 - 28
M A A S A I	430	502	73	274
	301	430	602	502
	251	334	602	334
	232	376	502	376
Mean	304	411	445	372
M E R I N O	301	430	602	430
	201	251	215	251
	251	334	376	334
	188	301	274	274
Mean	235	329	367	322
Significance	N/S	N/S	N/S	N/S

TABLE 10

Relationship between egg output and red cell loss in four Red Maasai and four Merino lambs given a primary infection with H. contortus and challenged 100 days later.

	Average daily egg output (x 10^3)	Average daily red cell loss in ml	Average eggs/ml red cells (x 10^3)
Primary Infection :			
Maasai	623.8	7.34	84.98
Mean	±120.55	±0.74	
Merino	1,367.8	10.98	120.91
Mean	±374.84	±1.70	
Challenge :			
Maasai	86.2	2.40	35.87
Mean	±41.95	±0.25	
Merino	946.4	7.87	120.22
Mean	±283.42	±1.37	

TABLE 11

Circulating blood and red cell volumes (in ml) of four Red Maasai and four Merino lambs 5 days before a primary infection with *H. contortus* shown with venous haematocrit and body weight for comparison.

		Body Weight in kg	PCV	Blood Volume	Red Cell Volume	Red Cell Vol/kg Body Wt
M A A S A I		52.5	34	3,097.30	1,053.08	20.06
		32.0	40	1,796.91	718.76	22.46
		23.0	44	1,253.21	551.41	23.97
		32.0	51	1,827.90	932.23	29.13
	Mean	34.9 ±6.25	42.3 ±3.57	1,993.83 ±390.78	813.87 ±111.49	23.91 ±1.92
M E R I N O		28.0	35	2,058.19	720.37	25.73
		32.3	35	2,114.46	740.06	22.91
		28.0	35	1,798.10	629.33	22.48
		34.0	32	2,068.39	661.89	19.47
	Mean	30.6 ±1.53	34.3 ±0.75	2,009.79 ±71.62	687.91 ±25.63	22.65 ±1.28
Significance		N/S	N/S	N/S	N/S	N/S

TABLE 12

Circulating blood and red cell volumes (in ml) of four Red Maasai and four Merino lambs 88 days after a primary infection with ·H. contortus shown with venous haematocrit and body weight for comparison.

		Body Weight in kg	PCV	Blood Volume	Red Cell Volume	Red Cell Vol/kg Body Wt
M A A S A I		52.5	32.5	3,035.79	986.79	18.79
		42.0	33.5	1,795.33	601.44	14.32
		28.0	30.5	2,019.36	615.91	22.00
		39.0	29.5	1,659.91	489.67	12.56
	Mean	40.4 ±5.04	31.5 ±0.91	2,127.60 ±311.67	673.45 ±108.15	16.92 ±2.14
M E R I N O		37.0	30.5	2,035.93	620.96	16.78
		40.0	28.5	1,827.31	520.78	13.02
		34.0	28.5	1,750.43	498.87	14.67
		39.0	24.5	1,851.25	453.56	11.63
	Mean	37.5 ±1.32	28.0 ±1.26	1,866.23 ±60.52	523.54 ±35.36	14.03 ±1.11
Significance		N/S	N/S	N/S	N/S	N/S
Significance between primary infection and challenge						
Maasai		N/S	⊕	N/S	N/S	N/S
Merino		⊕	⊕	N/S	⊕	⊕

TABLE 13

Comparison of mean length of prepatent period and epg of four Red Maasai and four Merino sheep given a primary infection (1°) and challenge (Ch) and a secondary infection (2° - data from previous section) with H. contortus. The mean epg in each case is for the first 35 days of the infection.

		Maasai	Merino
Length of prepatent period in days:	1°	22.8 ±0.85	23.0 ±0.91
	2°	30.5 ±1.66	22.8 ±1.75
Epg:	1°	1,041 ±262.7	2,140 ±788.7
	Ch	125 ±43.5	2,333 ±490.6
	2°	500 ±246.3	2,032 ±510.6

TABLE 14

Comparison of Red Cell Loss, Red Cell Half-Life and PCV
in four Red Maasai and four Merino sheep given a primary
infection (1°) and challenge (Ch) and a secondary infection
(2° - data from previous section) with H. contortus.

			1°	Ch	2°	Significance
Red Cell Loss in ml:						
M A A S A I	1° v 2°	First 16 days of infection	2.9 ±0.84		2.7 ±0.70	N/S
		Days 17 - 35	7.8 ±1.52		7.6 ±2.20	N/S
	Ch v 2°			3.0 ±0.67	5.1 ±1.41	N/S
M E R I N O		First 16 days of infection	2.0 ±0.39		3.0 ±0.31	N/S
		Days 17 - 35	12.5 ±2.82		12.8 ±2.48	N/S
	Ch v 2°			7.8 ±2.98	7.6 ±1.31	N/S
Red Cell Half-Life in hours:						
MAASAI			289		223	N/S
MERINO			216		189	N/S
PCV:						
M A A S A I	1° v 2°	First 16 days of infection	32.3 ±2.37		26.3 ±1.69	N/S
		Days 17 - 35	24.6 ±0.45		18.9 ±1.39	⊕ P<0.01
	Ch v 2°			31.2 ±1.10	22.8 ±1.52	⊕ P<0.01
M E R I N O	1° v 2°	First 16 days of infection	27.3 ±1.92		25.5 ±1.83	N/S
		Days 17 - 35	19.7 ±0.64		13.9 ±1.05	⊕ P<0.01
	Ch v 2°			28.3 ±2.50	20.0 ±1.47	⊕ P<0.05

DISCUSSION

A comparison between the pathophysiology of this primary infection with Haemonchus in Red Maasai and Merino lambs with that of the previously described secondary infection in experienced sheep shows marked similarities in their response to infection. This indicates that the superior resistance of the Maasai is innate, or at least rapidly acquired during the course of initial exposure to the parasite.

The most marked difference between the results of this trial and those of the last is that a difference in the clinical parameters of disease between the two breeds did not become evident until 10 days later in the primary infection than in the secondary infection. This is particularly marked in the red cell clearance in which a difference between the breeds occurred from day 16 in the secondary infection but not until day 26 in the primary infection. Similarly, the pattern of worm egg production is different in the primary and secondary infections. In the secondary infection patency in the Maasai sheep occurred considerably later than in the Merinos and the epg was then maintained at approximately one-tenth of that of the Merinos. In the primary infection however, patency occurred simultaneously in both breeds and the pattern of worm egg production did not separate until 15 days after patency when the Merino epg began to climb rapidly to a mean of 8,100 epg until just before challenge while that of the Maasai fluctuated around a mean of 1,000 epg for the first 60 days after infection and then began to

fall gradually to around 100 epg before challenge.

If epg and red cell loss are used as a measure of worm burden which in a primary infection is a fair assumption, (Roberts, 1957; Altaif, 1975) this suggests that in a primary infection, worm expulsion occurs after patency rather than just before patency as in secondary infections and takes place gradually over a period of several weeks rather than dramatically in a few days.

The fact that worm expulsion appears to occur later and less abruptly in a primary infection than in subsequent infections strongly suggests that the underlying mechanism is immunological, the primary infection inducing a primary response to the worms and sensitising the host so that future infections are rapidly expelled. To clarify this point more experiments need to be performed involving necropsies to assess worm burden and serological techniques to monitor the antibody and lymphocyte response in primary and secondary infections, but the evidence from this initial trial strongly implicates the immune system in worm expulsion. The difference therefore in the final worm burden in the Maasai and Merino sheep would be due to a difference in the efficiency of the immune response in these breeds to Haemonchus antigens.

It will be noted that in each breed a decline in epg occurred before challenge ie from day 60 in the Maasai and around day 90 in the Merinos. The decline in epg was not

therefore the result of challenge but of an increasingly

efficient immune reaction to the primary infection. After

challenge a slight increase in epg followed in each breed but

was succeeded by a marked drop six days after challenge

corresponding with the third to fourth larval stage moult and

self-cure of the previous infection as described by Stewart

(1950). As the new infection became patent the epg started to

rise once again, but at different times in each breed ie on day

29 of the challenge in the Maasai and 19 in the Merinos,

indicating a later patency date of the challenge infection in

the Maasai compared with that in the primary infection.

The later patency date of the challenge infection combined

with evidence of a decreased fecundity of the worms in the

Maasai lambs (as judged by eggs/ml red cells lost shown in

Table 10) is indicative of the development of additional acquired

immunological factors. Similarly Altaif (1975) reported the

presence of such phenomena as stunting, a longer prepatent

period and a decreased reproductive and haematophagic status

only in worms recovered after secondary infections and noted

the marked absence of such factors during primary infections.

The differences in mean PCV and daily red cell loss shown

in Table 14 between the secondary infection and challenge infec-

tion are particularly interesting since they indicate a lower

pathogenicity during a primary infection with a challenge

infection superimposed upon it than during a secondary infection

of experienced sheep. The reason for this is almost certainly

due to the removal of the antigenic stimulus of infection prior
to the administration of the experimental secondary infection,
the sheep having been drenched with anthelmintic and kept
worm-free for three months before the start of the experimental
infection.

There is considerable evidence that removal of many
helminth infections with anthelmintic results in a marked
decline of immunity to a subsequent infection with the same
parasite. Thus Altaif (1975) found no difference in worm
numbers in Scottish Blackface and Finn Dorset lambs between
a primary experimental infection and a subsequent infection
given only 18 days after the termination of the first infection
with anthelmintic. Since lambs of a similar age and breed were
subsequently shown to resist reinfection and expel an existing
worm population when a second infection was superimposed on
the first, Altaif concluded that the lack of resistance to
reinfection was due to the removal of the antigenic stimulus of
the parasite rather than to other factors such as age or
immunological exhaustion. Similarly Benitez-Usher (1975) has
shown that the successful immunising schedule of Jarrett and
Urquhart and their colleagues (Jarrett, Jennings, McIntyre,
Mulligan and Sharp, 1961; Urquhart, Jarrett and Mulligan,
1962; Lopez and Urquhart, 1967) using irradiated Haemonchus
larvae breaks down when the larvae are removed by anthelmintic
treatment, and Roberts and Keith (1959) and Ross (1963) have
shown a similar decreased immunity to reinfection with H. placei
in calves given anthelmintic.

SUMMARY

Four Red Maasai and four Merino lambs aged 10 - 13 months which had been raised worm-free were given an experimental primary infection of H. contortus and challenged 100 days later. Their response to infection was monitored using ^{51}Cr labelled erythrocytes, haematocrit, worm egg production and changes in body weight. Necropsies were not performed.

The response of the lambs to primary infection was very similar to that reported in the previous section and breed differences were marked but did not become evident until 26 days after infection - 10 days later than in the secondary infection. The pattern of worm expulsion as judged by the epg appeared to be gradual rather than sudden and was most marked from day 60 in the Maasai and around day 90 in the Merinos. It was suggested that worm expulsion is an immune phenomenon, the slower expulsion in the primary infection representing a primary immune response to infection. The effect of challenge was to cause a drop in the epg 6 days later representing self-cure of the primary infection followed by a rise in epg as the challenge infection became patent.

Patency of the primary infection occurred simultaneously in both breeds but that of the challenge infection was 10 days later in the Maasai than the Merinos. This delayed patency along with evidence of decreased fecundity of worms in the Maasai was interpreted as evidence of the development of further

102

acquired immunity by the Maasai lambs.

THE INFLUENCE OF BREED ON THE RESPONSE OF SHEEP TO HIGH INFECTION LEVELS WITH HAEMONCHUS CONTORTUS

Recent work on resistance to H. contortus linked to haemoglobin type in Scottish Blackface and Merino sheep (Altaif, 1975; Altaif and Dargie, 1976; Allonby J, personal communication) has shown that although clearly operative at low infection levels, the observed difference in response of haemoglobin A (HbA) and haemoglobin B (HbB) sheep is obliterated by high challenge.

In Scottish Blackface sheep Altaif (1975) and Altaif and Dargie (1976) found that significant differences both in establishment of worms and the consequent pathogenicity of infection occurred between individuals of different Hb types at infection levels of 350 infective larvae per kilogram body weight (350 L3/kg). However, upon infection with 1,400 L3/kg the superior resistance to infection of individuals with HbA alleles was no longer evident, there being no significant differences in worm numbers or the severity of disease between sheep bearing alleles for HbA or HbB.

In Merino sheep however, Allonby (J, personal communication) has shown that Hb linked resistance breaks down at a much lower threshold of 100 - 150 L3/kg and indeed that a difference in the response of HbA and HbB sheep is only marked at very

104

low infection levels of 25 - 50 L3/kg.

In view of the important bearing that challenge levels may have upon resistance to H. contortus in the field and the fact that this is clearly a restricting factor in haemoglobin-linked resistance, it was decided to investigate the response of Red Maasai and Merino sheep to a severe experimental infection of H. contortus . The infective dose chosen was 1,000 L3/kg as being the highest likely to be encountered under field conditions.

METHODS AND MATERIALS

Two experiments were conducted involving sheep
previously exposed to infection with H. contortus. In the first
experiment 8 sheep were used - 4 Red Maasai (2 females,
2 males) and 4 Merinos (2 females, 2 males) and 13 sheep in
the second experiment - 6 Red Maasai (1 female, 5 wethers)
and 7 Merinos (5 females, 2 wethers). Ages ranged from
1 to 8 years at the time of infection; all were of haemoglobin
type B. The sheep were drenched with thiabendazole at
100 mg/kg one week prior to infection per os with a single
dose of 1,000 infective larvae of H. contortus per kilogram
body weight, cultured from faeces of an experimentally
infected Merino wether. Once a week the sheep were weighed
and twice a week haematocrit was measured and faecal samples
taken per rectum for estimation of worm eggs per gram of faeces
(epg) by the modified McMaster technique. In both experiments
the sheep were kept indoors and given Rhodes hay, concentrates
and water ad lib. Their enclosure was cleaned out every other
day to prevent reinfection. In the second experiment 3 Maasai
and 2 Merino wethers were necropsied 10 weeks after infection.
No other necropsies were performed.

RESULTS

In each experiment the general pattern was the same -
namely a falling haematocrit in the Merinos while that of the
Maasai remained largely unchanged and a worm egg count
which in the Maasai remained steadily between 200 and 600 epg
while that of the Merinos rose rapidly to a mean peak of
40,000 epg during the 6th to 7th week of infection, falling and
fluctuating erratically thereafter. This is illustrated
graphically in Figures 12 and 13. The prepatent period of the
infection was longer in the Maasai (33 ± 3 days) than the
Merinos (27 ± 3 days) in both cases.

In the first experiment both male Merinos showed evidence
of clinical haemonchosis, becoming progressively weaker and
dying - one during the 6th week of infection and the other a
week after drenching with anthelmintic at the end of the
experiment, while the two female Merinos showed no overt signs
of clinical haemonchosis. There was no difference in epg
between the sexes in the Maasai breed (female mean 306 ± 113,
male mean 274 ± 68) but a very marked difference in the Merinos
(female mean 1,257 ± 437, male mean 20,463 ± 5,594). Since
no sex differences were noted in the second experiment, for
clarity a mean epg value has been taken for both sexes in each
experiment.

In the second experiment one female Merino died during
the 10th week of infection and 3 Maasai wethers and the 2 Merino

wethers were autopsied two days later to assess worm burden.
The worm burdens, shown in Table 15 are considerably lower
than those obtained earlier from the lower infection rate of
350 L3/kg where the mean worm burden for Maasai killed
between 18 and 28 days after infection was 224 ± 83 compared
with 67 ± 44 in this experiment and similarly in the Merinos,
2,872 ± 384 compared with 690 ± 250. Expressed in terms of
the percentage of the infective dose which was recovered at
necropsy this represents a difference between 4% and 0.14% in
the Maasai and 37% and 2.21% in the Merinos. Worm burdens
were not assessed in animals which died since total or partial
expulsion of the worm burden is known to occur just prior to
death from haemonchosis (Allonby, personal communication).

In the first experiment the haematocrit in both breeds shows
the first two stages of the classic three stage pattern described by
Dargie and Allonby (1975) of a rapid initial fall levelling out and
then either remaining steady or in this case actually increasing
slightly, which represents the lag phase, stimulation and finally
the response of the erythropoietic system to haemorrhage. The
final, exhaustion stage was not reached due to the termination of
infection with anthelmintic prior to its onset. In the Maasai there
was a net fall of 2% in their PCV and a net fall of 5% in that of the
Merinos. In the second experiment the pattern was not so clear,
but the Maasai again suffered a net fall of 2% in their PCV and
the Merinos a fall of 9% from their initial PCV.

The change in body weights of the two breeds during

infection was significant only in the second experiment where the Merinos lost a mean of 11.1% of their initial body weight (P<0.01) as compared with 7.4% in the Maasai (P<0.05).
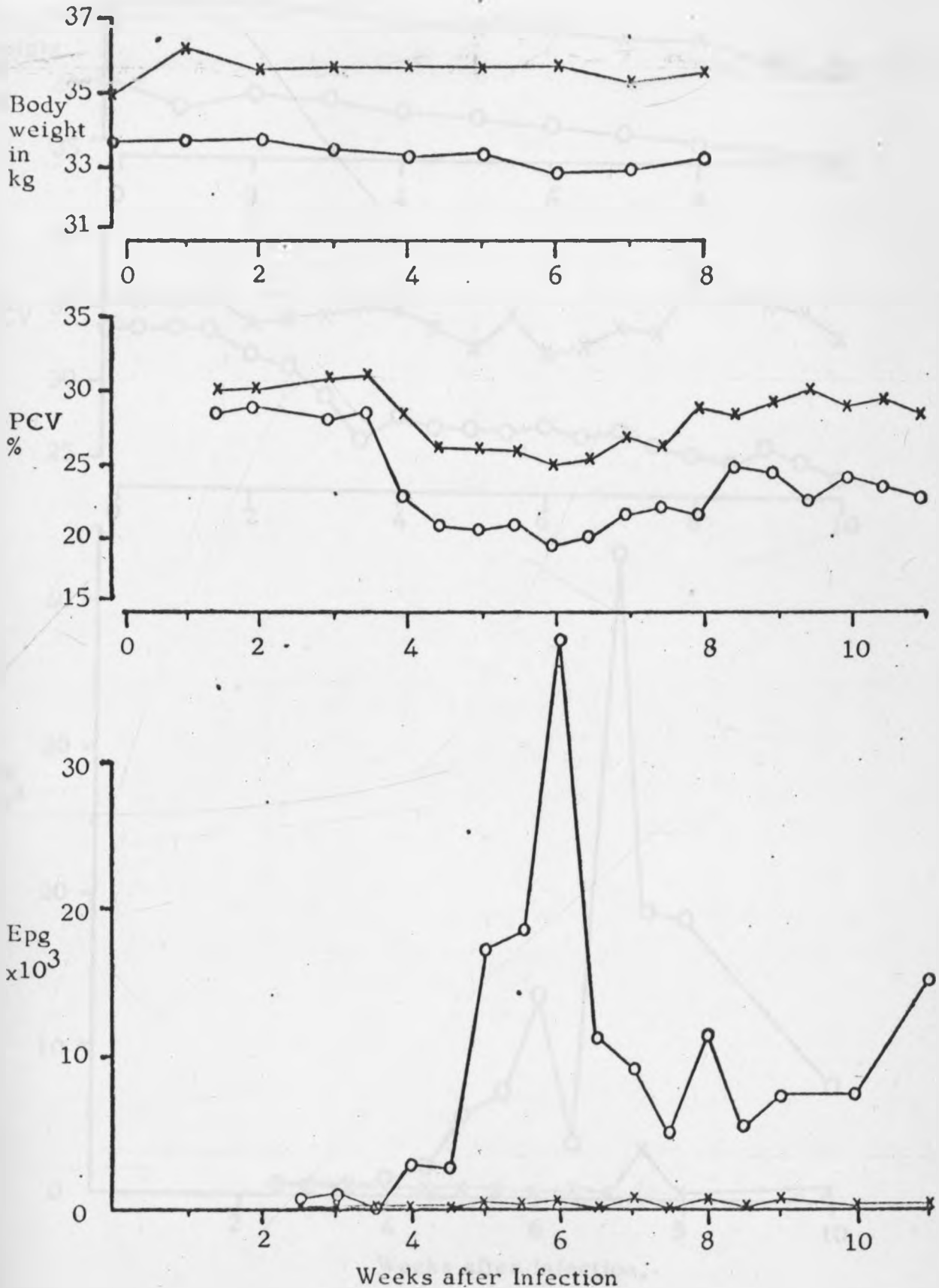
Figure 12: The mean body weight, haematocrit and faecal egg count of four Red Maasai (x-x) and four Merino (o-o) sheep given an experimental infection of H. contortus of 1,000 L3/kg
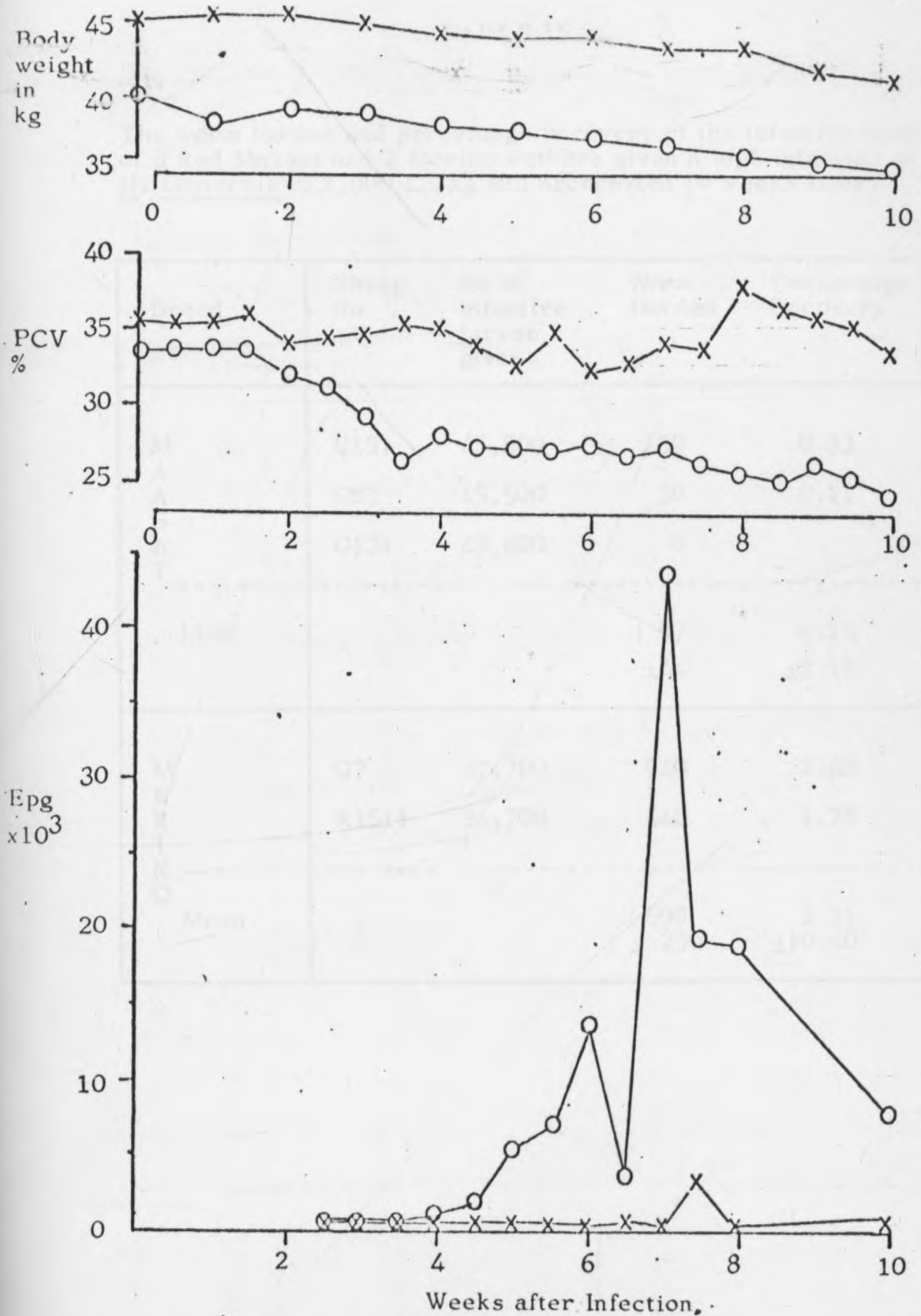
Figure 13: The mean body weight, haematocrit and faecal egg count of six Red Maasái (x-x) and seven Merino (o-o) sheep given an experimental infection of H. contortus of 1,000 L3/kg

TABLE 15

The worm burden and percentage recovery of the infective dose
of 3 Red Maasai and 2 Merino wethers given a high infection of
H. contortus of 1,000 L3/kg and necropsied 10 weeks later.

Breed	Sheep No	No of infective larvae given	Worm Burden	Percentage Recovery
M A A S A I	U151	45,500	150	0.33
	G82	45,500	50	0.11
	G131	49,400	0	-
Mean			67 ±44	0.14 ±2.16
M E R I N O	G7	37,700	940	2.49
	R1511	24,700	440	1.78
Mean			690 ± 250	2.21 ±10.40

DISCUSSION

From the results of this experiment it is clear that even at high levels of infection, the Maasai sheep retain their superior level of resistance, expelling the vast majority of their worms and thus minimising the pathogenesis of the infection. The response of the Merinos however was typically variable, some individuals producing vast numbers of worm eggs and rapidly succumbing to the infection while others maintained a very low egg count throughout and showed no obvious signs of disease. This is well illustrated in the first of the two experiments in the response of the two male and two female Merinos to infection. Both males died as a result of the infection while the two females showed few signs of disease, one of them maintaining a very low egg count throughout and the other having no detectable eggs in its faeces until 42 days after infection but then establishing a high epg.

A superior resistance to infection by the female Merinos did not however occur in the second experiment, the only fatality being a female and no significant differences were detected in the faecal egg count or degree of anaemia between the sexes. It is not therefore accurate to describe the level of response as being sex-linked and response to infection is probably related more to the genetic make-up of the sheep concerned than to their sex.

Such individual variations apart, the mean overall resistance

of the Merinos to a high challenge infection of H. contortus
is very much lower than that of the Red Maasai as measured by
worm numbers recovered at autopsy, epg and the degree of
anaemia of the disease. Although only three fatalities occurred
in the eleven Merinos infected, it is worth speculating how
many more may have occurred had the infection not been
artificially terminated. Certainly at the end of the first
experiment one female had an epg comparable with that of the
two males which died and at the end of the second experiment
both males and two females had similarly high egg counts.
Plainly the vast majority of the Merinos were experiencing
severe chronic haemonchosis as described by Dargie and
Allonby (1975) and it was probably only a matter of time before
their erythropoietic systems became exhausted and,unable to
compensate further for the continuous haemorrhage,they too
would have died.

The very low worm recoveries from the five sheep necrop-
sied is surprising in view of the large size of the infective dose
and the larger recoveries described in Section 1 from a much
smaller infective dose. The explanation could be that fewer
worms initially established which is quite likely and may be
related to the very large size of the infective dose, or that
a comparable number of worms established but were later
expelled. Without doing serial kills and worm counts during the
early part of the infection it is not possible to distinguish between
a lower initial establishment rate and a more effective prepatent
expulsion phase.

The abrupt drop in epg after the seventh week of infection may represent a further period of worm expulsion and the gradual increase in epg thereafter seen in several individuals and also in the mean Merino epg in the first experiment, might represent either a breakdown in the acquired immunity of the host which was previously limiting the fecundity of the worms or more likely, the attainment of sexual maturity by worms previously inhibited as fourth-stage larvae.

The latter explanation seems the more probable in view of the observations of Blitz and Gibbs (1972a) that the percentage of inhibited larvae is directly proportional to worm burden. Whether larval inhibition is related to inter-worm competition or a host response is debatable. Dineen and Wagland (1966a) suggest that all effects upon the parasites are solely due to the host reaction, but Michel (1963) reported that in Ostertagia ostertagi in calves, removal of the adult worm population with anthelmintic resulted in the resumption of development by the inhibited larvae, suggesting some inter-parasite reaction related to the number of worms present. Michel further suggested that there is a population turnover due to loss of adults and development of inhibited larvae. If a similar system is operating in this case, this would explain the sudden fall in epg around week 7 and the subsequent gradual rise in some individuals as inhibited larvae resumed their development and began to produce eggs. Unfortunately differential worm counts were not performed so it is not possible to estimate the propor-tion of inhibited fourth-stage larvae in the recovered worm

populations.

SUMMARY

In two experiments a total of 10 Red Maasai and 11 Merino sheep were given a single dose of 1,000 infective larvae per kilogram body weight of H. contortus to determine whether breed resistance in these breeds is subject to the same restrictions of infection level as haemoglobin-linked resistance to H. contortus.

The criteria used were a weekly weight change, a biweekly haematocrit and epg and an autopsy of 5 animals at the end of the second experiment. The results indicated an unbroken resistance by the Maasai as shown by a steady haematocrit, very low epg and worm burden one-tenth the size of the Merinos'. The Merinos included 3 fatal cases of haemonchosis, a generally very high epg and falling haematocrit but the individual variation was large. The very low worm recoveries in both breeds was suggested to be due either to a lower rate of establishment or a higher rate of worm expulsion at patency.

THE INFLUENCE OF BREED ON THE RESPONSE OF GOATS TO EXPERIMENTAL INFECTION WITH HAEMONCHUS CONTORTUS

In many tropical and subtropical countries goats are at least as important and often more important than sheep as a source of protein to pasturalists and small-scale farmers. The goat is predominantly a browser rather than a grazer and is thus more suited to marginal zones where except for a short period each year after rain, grass and other herbs are unavailable and small stock must rely entirely upon xerophytic shrubs and small trees. In less arid areas goats and sheep are commonly herded together so that their feeding activities complement each other and maximum use can be made of available forage. The importance of the goat to pasturalists, particularly those which lead a nomadic way of life can hardly be overstated and indeed the possession of goats in many tribes has become a measure of wealth and status as much as an insurance for the future.

The goat like the sheep is also a natural host to H. contortus (Soulsby, 1965; Dunn, 1969) which it may harbour in comparable numbers and frequency to sheep (LeJambre and Royal, 1976; personal observation). There is also some evidence that goats are more susceptible than sheep to infestation with gastro-intestinal nematodes when restricted to pasture alone (LeJambre and Royal, 1976). It is surprising therefore that so little attention has been paid to the role of H. contortus in goat husbandry and

to the host-parasite relationship involved.

For this reason a trial was designed to monitor the response of two breeds of goat to an experimental infection of H. contortus under field conditions. The study area chosen is typical of much of Kenya, being semi-arid scrub which is more suited to goat rather than sheep husbandry.

METHODS AND MATERIALS

The study was conducted at Kiboko near Makindu on the boundary of Kajiado and Machakos districts in a dry scrub area endemic for trypanosomiasis.

Two breeds of goat were used in the experiment, an exotic/ indigenous cross, the Saanen/Galla and a local breed, the Small East African. A total of 40 goats were used, 20 of each breed, of which 10 were infected with II. contortus and 10 were left uninfected as controls. The goats were all adults of haemoglobin type B which had been bred and reared in the area.

All animals were treated with Nilverm (ICI Macclesfield, England) and Berenil (Hoechst, Germany) before the start of the experiment to remove any existing worms or trypanosomes and sprayed with acaricide twice a week during the course of the study. Thick blood films were made once a week and any animals with trypanosomes were again treated with Berenil. The goats were kept out of doors throughout the trial and herded into enclosures at night.

Five goats from each of the four groups were used to study red cell half-life. Approximately 50 ml of blood were withdrawn from the jugular vein of each goat into a heparinised container, centrifuged and the red cells washed in physiological saline and incubated with 1 ml ^{51}Cr as sodium chromate for 30 minutes at 37°C as previously described. After labelling, the cells were

washed three times in saline, reconstituted with their own plasma and injected via a jugular catheter back into their donors. Fifteen minutes were allowed for complete mixing and a 1 ml sample taken from the opposite jugular vein. Each day thereafter a carefully measured 1 ml blood sample was diluted in 9 ml of 0.05% NaOH and all samples were counted in a gamma scintillation counter on the same day at the end of the experiment. The red cell activity of the 15 minute sample was calculated from the PCV and each daily sample expressed as a percentage of this and from this the red cell half-life was calculated as before. Six weeks after infection each goat was given more ^{51}Cr labelled red cells in the same way in order to estimate red cell half-life for the final stage of infection.

The goats were infected orally with H. contortus seven days after isotopic labelling at a rate of 250 infective larvae per kilogram body weight. The larvae were cultured from a Merino wether at Kabete experimentally infected with the Merino strain of H. contortus.

Faecal samples were taken per rectum twice weekly for estimation of epg by the modified McMaster technique. All animals were necropsied 13 weeks after infection and their abomasa were removed., washed, scraped and subjected to pepsin digest as already described and the worm burden assessed from a 20% aliquot.

EXPERIMENTAL DESIGN

Week	Treatment	Saanen/Galla				Small East African			
		Infected		Control		Infected		Control	
		10		10		10		10	
		5	5	5	5	5	5	5	5
0	^{51}Cr	+	-	+	-	+	-	+	-
1	H. contortus	+	+	-	-	+	+	-	-
7	^{51}Cr	+	-	+	-	+	-	+	-
14	Necropsy	+	+	+	+	+	+	+	+

The Saanen/Galla goats showed a significantly lower establishment of worms and a lower absolute worm burden than the Small East African goats but, surprisingly their epg was higher with a mean of $2,349 \pm 409$ compared with $1,557 \pm 284$, (**Table 16**).

The epg of both breeds showed a very similar pattern, the infection becoming patent on day 21 in the Saanen/Galla and on day 24 in the Small East African, reaching a maximum at 6 weeks after infection (of 6,360 in the Saanen/Galla and 4,250 in the Small East African goats), remaining high from 7 to 11 weeks fluctuating around a mean of 4,500 in the Saanan/Galla and 2,500 in the Small East African and decreasing thereafter. This is illustrated in Figure 14.

The red cell half-life in the two breeds was calculated separately for three stages in the infection, the first between 0 and 3 weeks after infection being the prepatent period, the second from 4 to 5 weeks being the patent and the third from 7 to 8 weeks being the chronic stage of infection. These values are shown in Table 17. No significant differences were recorded between the uninfected controls of each breed or between the Saanen/Galla and their controls. In the Small East African goats there was a significant difference between the infected and uninfected groups during the second and third stages (Table 18) and also between the two breeds during the second stage of infection (Table 17).
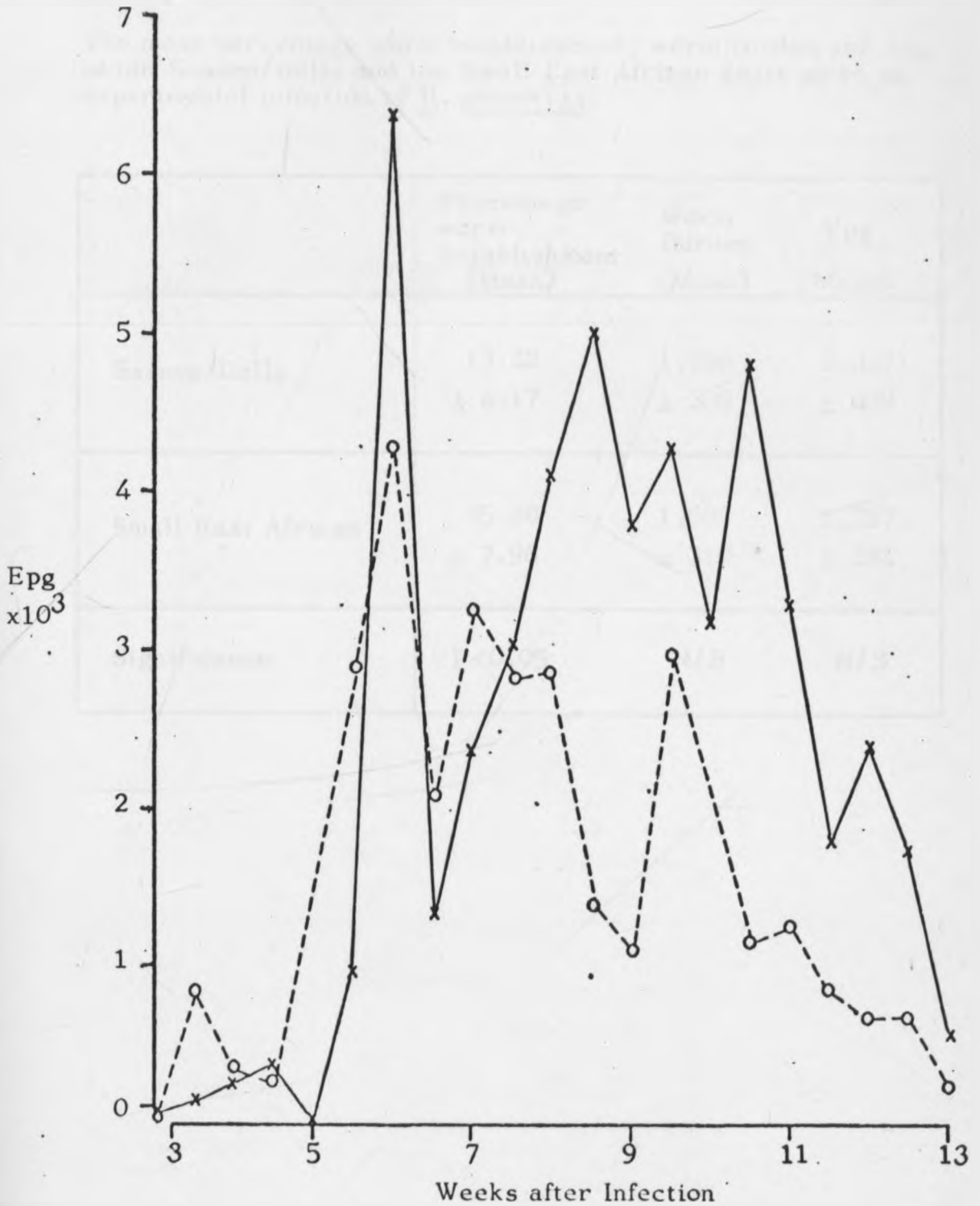
Figure 14: The mean faecal egg count of ten Saanen/Galla(x-x) and ten Small East African (o-o) goats given an experimental infection of H. contortus

TABLE 16

The mean percentage worm establishment, worm burden and epg of ten Saanen/Galla and ten Small East African goats given an experimental infection of H. contortus.

	Percentage worm Establishment (Mean)	Worm Burden (Mean)	Epg (Mean)
Saanen/Galla	13.42 ± 4.17	1,196 ± 339	2,349 ± 409
Small East African	35.20 ± 7.96	1,602 ± 310	1,557 ± 284
Significance	P<0.05	N/S	N/S

TABLE 17

The mean red cell half life ($t_{\frac{1}{2}}$) in hours of five Saanen/Galla and five Small East African goats given an experimental infection of H. contortus compared with the $t_{\frac{1}{2}}$ of five uninfected controls of each breed.

Weeks after Infection		Saanen /Galla	Small East African	Significance
0 - 3	Infected	332	324	N/S
4 - 5	Infected	332	201	P<0.05
7 - 8		205	184	N/S
0 - 3	Controls	353	379	N/S
4 - 5	Controls	353	379	N/S
7 - 8		255	284	N/S

TABLE 18

Significance in red cell half-life between infected and control Saanen/Galla and Small East African goats.

Weeks after Infection	Saanen/Galla	Small East African
0 - 3	N/S	N/S
4 - 5	N/S	P<0.005
7 - 8	N/S	P<0.05

DISCUSSION

The marked difference not only in actual worm burden but in the percentage of the infective dose which was recovered from the two breeds of goat indicates a clear-cut difference in the response of the breeds to infection with Haemonchus contortus.

One surprising fact was that it was the exotic/indigenous cross breed rather than the indigenous breed which was the more resistant to Haemonchus. This is a reversal of the more common situation in which the indigenous rather than the exotic individuals are more resistant to local parasites as shown by the results presented in the previous sections of this thesis and also by the work of Bradley, Loggins and their co-workers (1965, 1972, 1973); Knight, Vegors and Glimp (1973) and Patnaik, Mathur and Pachalag (1973). However it is important to bear in mind that resistance to haemonchosis may not be the most important survival factor in an area of limited rainfall which is furthermore endemic for trypanosomiasis.

The Small East African goats were approximately half the size of the Saanen/Galla and because the number of infective larvae given to each animal was based on its weight, received about half the number of infective larvae given to the Saanen/Galla. However, since significantly more larvae established in the Small East African breed, they were found at necropsy to actually harbour more worms than the Saanen/Galla.

As a direct result of this difference in worm burden, the red cell half life of the infected Small East African goats was shorter than that of the Saanen/Galla and also shorter than that of the uninfected Small East African goats (Tables 17 and 18). In view of the fact that the red cell half-life of the control Small East African goats was actually longer than that of the Saanen/Galla, this represents a large difference in red cell survival. In contrast, the red cell half-life of the infected Saanen/Galla was at no stage significantly different from that of their uninfected controls.

The slightly earlier patency date and higher mean epg of the Saanen/Galla which is not related to worm burden is difficult to interpret. However, an explanation may be offered if we assume that breed differences are due to immunological factors, some of which effect worm expulsion and some of which act upon the remaining population. In other words, less worms actually persist in the Saanen/Galla due to an immune factor acting during the early part of the infection, but those worms which do establish thrive, while more worms establish in the Small East African goats but having done so are acted upon by other immunological factors which delay patency and reduce their subsequent fecundity.

This would then appear to be a reversal of the situation already described in the Red Maasai and Merino sheep, whereby the indigenous sheep are able to both expel the vast majority of H. contortus worms and severely restrict the fecundity of the

residual population while the exotic breed has a much weaker
expulsive mechanism and a very variable level of acquired
immunity.

SUMMARY

Ten Saanen/Galla and ten Small East African goats were given an experimental infection of <u>Haemonchus</u> <u>contortus</u> at 250 L3/kg body weight. The goats were all adults of Hb type B which had been reared in the study area which is a semi-arid area of dry scrubland endemic for trypanosomiasis. The goats were drenched with anthelmintic before the start of the experiment and kept free of ticks and trypanosomes throughout the course of the experiment. Response to infection was monitored using ^{51}Cr labelled red cells to estimate red cell half-life, epg and worm burden at necropsy, and results from infected individuals were compared with those from ten uninfected controls of each breed.

Worm burdens were considerably higher and red cell half-lives were considerably lower in the indigenous breed although epg was higher and patency earlier in the exotic/indigenous cross. It was suggested that this was related to a difference in immune factors in the two breeds, the Saanen/Galla being more efficient at limiting the establishment of <u>H</u>. <u>contortus</u> than the Small East African goats as judged by worm numbers, while the indigenous goats have a higher level of other immune factors which restrict the fecundity of the remaining worms.

DISCUSSION

In view of the importance of Haemonchus in the sheep and
goat industry and the lack of success of vaccination schedules in
lambs, a better understanding of natural immunity is imperative,
particularly where such knowledge may be applied to preventive
measures. The natural difference in helminth resistance in two
breeds of sheep offers an ideal model in which to investigate more
closely the individual components of immunity, particularly where
the breed difference is marked as in the Red Maasai/ Merino
model.

The Red Maasai breed is particularly remarkable in its
solid resistance to Haemonchus, indeed to my knowledge, no
other breed of sheep has been described which approaches the
Red Maasai in this respect and for this reason the Red Maasai
would make excellent candidates for further work on immunity
to helminths.

That the resistance of the Red Maasai sheep to H. contortus
is an immune phenomenon rather than a physiological difference
between the breeds is supported by the occurrence of effects
upon the parasites which are generally held to be due to
physiological damage caused by immune factors such as stunting,
delayed patency and impaired reproductive potential.
Furthermore, the delay in response of the Maasai sheep during
a primary infection is highly suggestive of a primary response
to an antigenic stimulus so typical of other models.

In the Red Maasai sheep resistance to Haemonchus is not
only pronounced but also very predictable in its nature,
developing rapidly during a primary infection, remaining
unbroken by heavy challenge and also in ewes lambing during a
primary infection. This was a surprising observation in view
of the well documented effects of parturition and lactation upon
resistance to helminths and to Haemonchus in particular where
lambing is usually followed by a marked rise in egg output -
the so-called 'spring rise' phenomenon (Connan, 1968; Proctor
and Gibbs, 1968; O'Sullivan and Donald, 1970; 1973; Blitz and
Gibbs, 1972b). In contrast the Merino resistance was in general
much weaker and very variable in its nature, individuals ranging
from highly susceptible to highly resistant.

The essential difference in the response of these two breeds
of sheep to infection with H. contortus revolves around the
efficiency of worm expulsion rather than worm establishment or
subsequent compensation for worm effects. This is well illus-
trated in the first section of this thesis where worm burdens
recovered during serial kills were very similar in both breeds
until a point between 16 and 18 days after infection when both
breeds underwent a phase of worm expulsion which in the Maasai
resulted in a reduction to a mean worm burden of 4% of the
infective dose but to only 37% in the Merinos.

A very similar pattern of expulsion was reported in Finn
Dorset and Scottish Blackface sheep by Altaif (1975) and Altaif
and Dargie (1976) who suggested that the stimulus for expulsion

was the fourth to fifth larval stage moult which occurs 9 - 11
days after infection (Soulsby, 1965). The delay between this
stimulus and worm expulsion is likely to be due to the time
taken for antigens released during the moult to be processed
and the actual expulsion mechanism is then probably an allergic
reaction as described by Stewart (1953) involving histamine and an
oedematous reaction in the abomasal mucosa. Blood histamine
was not monitored during this study but the abomasum of one
of the Maasai sheep killed 15 days after infection was observed
to be swollen, oedematous and suffused with blood giving it a
dark purple colour while those recovered at other times were
pale and flaccid. The abomasum of the other Maasai killed on
the same day showed similar changes but to a lesser extent while
those of the two Merinos appeared to be normal.

Worm expulsion, which is the vital factor in the sheep/
Haemonchus relationship is still incompletely understood, but
appears to be a highly complex mechanism involving cellular as
well as humoral factors and triggered first by a specific antigen-
antibody (or sensitised lymphocyte) reaction and then effected by
a non-specific amine or lymphokine mediated reaction.

The generalised sequence of events leading to worm expulsion
seems to be firstly an afferent stage involving the interaction of
parasite antigens with specific immunoglobulins attached to the
surface of lymphocytes, causing the lymphocytes to divide and give
rise to progeny which then differentiate into effector cells
producing either circulating immunoglobulin (which is predominantly

IgG) or lymphokines. This is followed by a specific interaction
of the immunoglobulin or sensitised cells with parasite antigen at
the site of infection and initiates the effector stage which is
thought to be non-specific. During the effector or efferent stage
amines (of which histamine and 5-hydroxytryptamine or 5-HT are
the most important) are released either directly by IgE-mediated
mast cell degranulation or indirectly by way of lymphokines.
These amines cause an allergic reaction in the tissues
surrounding the parasite making conditions unsuitable for the
worm and thus bring about its death or expulsion (Kelly, 1973).

Evidence for the importance of histamine in worm expulsion
was first presented by Stewart in 1953 while working on infections
of H. contortus and Trichostrongylus spp in sheep. Stewart
noted that 'self-cure' was normally accompanied by a rise in
blood histamine levels and that antihistamine drugs usually pre-
vented the occurrence of self-cure but did not interfere with
the rise in specific serum antibody normally associated with the
self-cure reaction. Subsequent research has confirmed the
importance of histamine and 5-HT in the expulsion of other
nematodes such as Nippostrongylus brasiliensis in the rat
(Murray, Smith, Waddell and Jarrett, 1971).

The Nippostrongylus/rat model is one of the best understood
of the host-intestinal nematode associations and Murray and his
colleagues (Murray et al, 1971; personal communication) have
presented evidence that the allergic reaction associated with
worm expulsion may be an intermediary step rather than the

effector mechanism itself. They noted a marked rise in the mast cell population of the intestinal wall and degranulation of these mast cells, probably due to an allergen-reaginic antibody reaction before worm expulsion occurred. Murray and his co-workers have shown that the amines released promote a hyperpermeability of the intestinal wall and increased macro - molecular leakage into the gut thus allowing direct contact between the parasites and large quantities of anti-worm antibodies. Prior to mast cell discharge and separation of the intestinal epithelial cells, some limited worm expulsion occurs and this is believed to be due to small amounts of antibody diffusing through the cytoplasm of the epithelial cells.

Although worm expulsion plays the major role in the sheep/ Haemonchus relationship, worm expulsion is very rarely complete and a residual population of worms is left behind. This residual population in its turn elicits other immune factors which act upon the remaining worms adversely affecting their growth and reproductive potential. This was well illustrated in Section One of this thesis where worms recovered from Maasai sheep were found to be considerably smaller throughout the course of infection than those from Merino sheep and had a later patency date (day 28 as opposed to day 21) and once patent there was some evidence that each female worm produced fewer eggs than those in the Merinos.

Further investigations, particularly those involving the individual components of the immune reaction in the Red Maasai

breed of sheep could prove most enlightening particularly
if conducted simultaneously in a more susceptible breed such
as the Merinos. A start may be made by monitoring circulating
eosinophils and immunoglobulin levels of the different immuno-
globulin classes during the course of infection; abomasal pH and
electrolytes and if possible, serial biopsies of the abomasal
mucosa taken via a cannula. Such parallel observations made
during a single infection could provide much valuable informa-
tion on the timing and extent of the various components of the
immune response. Abomasal cannulation is a relatively simple
operation (personal observation) and does not appear to interfere
with the course of an infection with H. contortus (Allonby,
personal communication) but the effect of repeated mucosal
biopsies on an infection is unknown.

The role of the various immunoglobulin classes in immunity
to helminths is still unclear. The main classes known to be
involved are IgE which adheres to mast cells and upon contact
with a specific worm antigen triggers mast cell degranulation
(Kelly, 1973), and IgG particularly IgG_1 which can be purified
and successfully used in passive immunisation by serum transfer
(Kelly, 1973; Ogilvie and Love, 1974).

A recent and most interesting discovery has been the obser-
vation of specific anti-larval IgA antibodies in the abomasal
mucus of sheep hyperimmunised against H. controtus (Smith, 1977).
This discovery has been followed up by other workers in
Glasgow who have subsequently shown that resistance to

Haemonchus following vaccination in adult sheep is closely
correlated with serum IgG and mucosal IgA levels, being
significantly higher in resistant adults than in challenge control
or worm-free adults and immunologically unresponsive lambs
(Duncan, Smith and Dargie, in press).

Similarly much more needs to be known about the role of the
various cell types which invade the mucosa during and prior to
worm expulsion. Of particular interest is the role of the
eosinophil which occurs in large numbers in the mucosal infiltrate.
The eosinophils may merely be 'by-stander' cells attracted to the
area by the release of amines or they may play a more active
part in worm expulsion. Of particular significance in this respect
is the discovery that eosinophils are the actual 'killer cells' in
schistosomiasis (Glauert and Butterworth, 1977) even though
the immune response directed against a tissue-embedded schisto-
somulum is likely to be different to that directed against a
lumen-dwelling intestinal nematode.

In view of the problems related to immunological unresponsive-
ness in the young ovine encountered by Urquhart and his colleagues
(Urquhart, 1964; 1970; Neilson, 1975) during attempts to
vaccinate Merino lambs with irradiated H. contortus larvae, it
would be worthwhile to conduct experimental infections in Maasai
neonates to establish the intensity and duration of this period in
a breed known to be highly resistant to infection. The primary
infection described in this thesis was deliberately designed to
exclude the unresponsive period in the Merinos on the assumption

that a similar or shorter period exists in the Maasai since the
more resistant Scottish Blackface breed investigated by
Christie and Brambell (1966) responded favourably to vaccination
at two and a half months of age compared with seven months in
the Merinos (Lopez and Urquhart, 1967).

Another interesting comparison between the Maasai and
Merino breeds could be made by monitoring the response of
these two breeds of sheep to antigens other than those of
Haemonchus to establish whether the superior immunity of the
Red Maasai to Haemonchus antigens also extends to other
unrelated antigens. This was actually attempted during the
course of this study but the results of the trial were equivocal
and time and funds did not permit the repetition of this
experiment.

In conclusion, breed resistance to Haemonchus contortus
in the Red Maasai and Merino breeds of sheep seems to be based
upon a difference between these breeds in the efficiency of
their immune systems to respond to Haemonchus antigens. This
immunity is expressed primarily by causing the expulsion of the
vast majority of the worms as they approach patency and
secondarily by restricting the reproductive activity of the
remaining worms. In contrast, haemoglobin-linked resistance
to infection with Haemonchus has been suggested to be largely
physiological in nature (Evans, Blunt and Southcott, 1963)
involving a possible greater availability to the worms of oxygen
and methionine in HbB hosts than in HbA hosts (Altaif, 1975;

Altaif and Dargie, 1976). Furthermore the fact that in the
Maasai breed of sheep resistance in not dose-dependent as in
haemoglobin-linked resistance in the breeds investigated,
namely Merino (Allonby J, personal communication) and
Scottish Blackface (Altaif, 1975; Altaif and Dargie, 1976)
confirms Altaif's (1975) conclusions that breed resistance is
superior to haemoglobin-linked resistance both in terms of the
degree of resistance engendered and in its independence from
the size of dose administered.

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